What kind of drugs should we develop

Mathematical and Computational Biology in Drug Discovery (MCBDD) Module III

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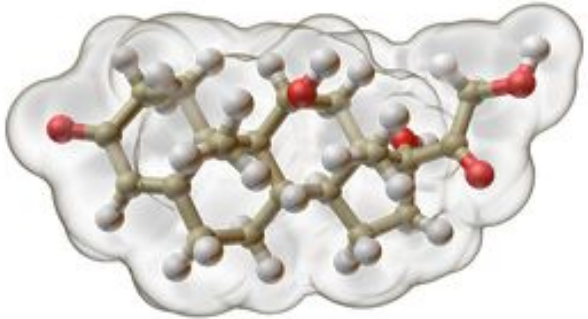
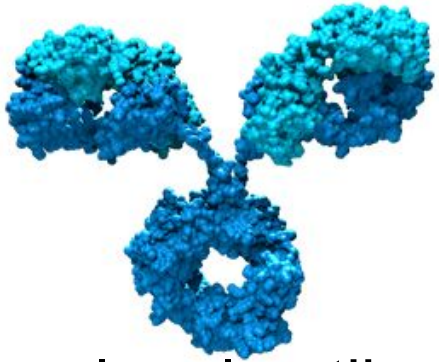
Overview

- Essentials of modalities
  - Small molecules: classical, protein degrader, RNA modulator
  - Large molecules: classical, DUTA-Fabs, protein design
  - Antisense oligonucleotides: siRNA, shRNA, ASO
  - Gene and cell therapy
- Three case studies:
  - Success stories:
    - [Small molecules] SMA (Evrysdi/Risdiplam and Nusinersen)
    - [Antisense] patisiran (KEGG DRUG) and givosiran (DrugBank, structure available at EMA)
    - [Offline read] mRNA vaccine (MIT Technology Review)
    - Turning failure into successes: [Multispecific drugs] Thalidomide, PROTAC, degraders
    - [Antibody] Cancer immunotherapy (CTLA4, PD1)
    - [Gene and Cell therapy] CAR-T
  - Challenges
    - [Antisense] HTT (Tominersen)
    - Difference between genetic and enzymatic inhibition

A zoo of modalities

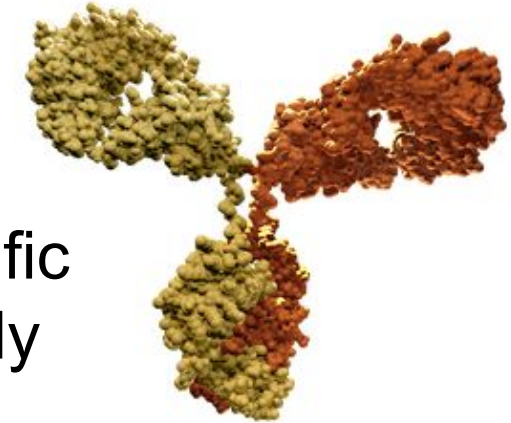


Small molecule

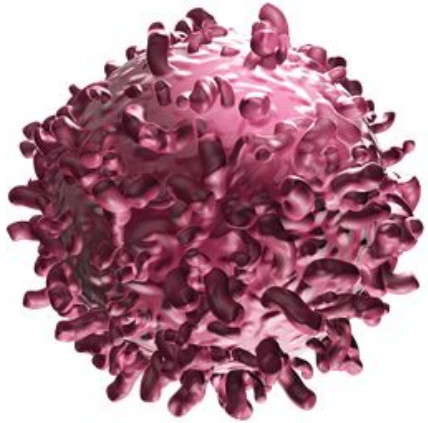


Monoclonal antibody

RNA inference

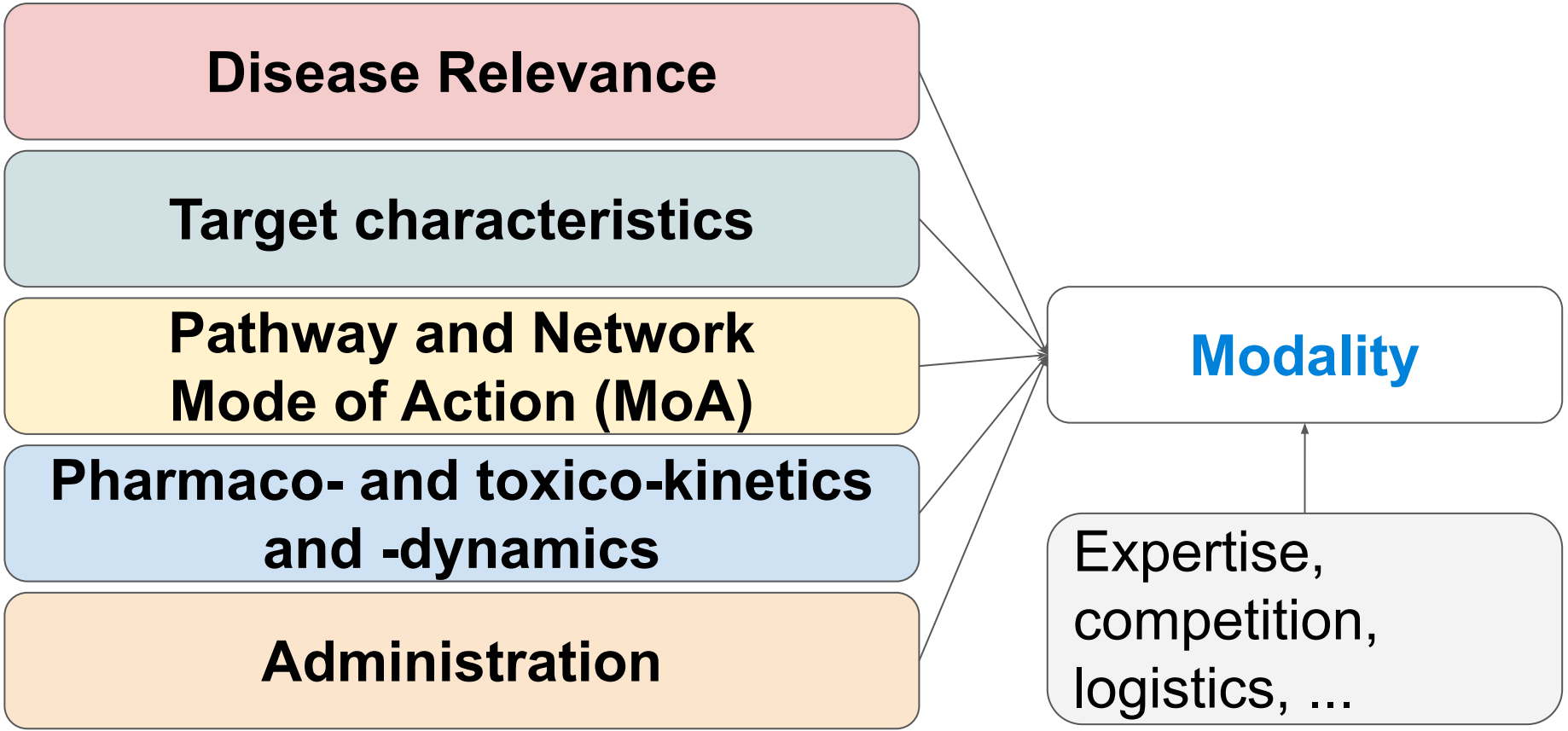


Bispecific antibody



Chimeric Antigen Receptor (CAR) T-cells

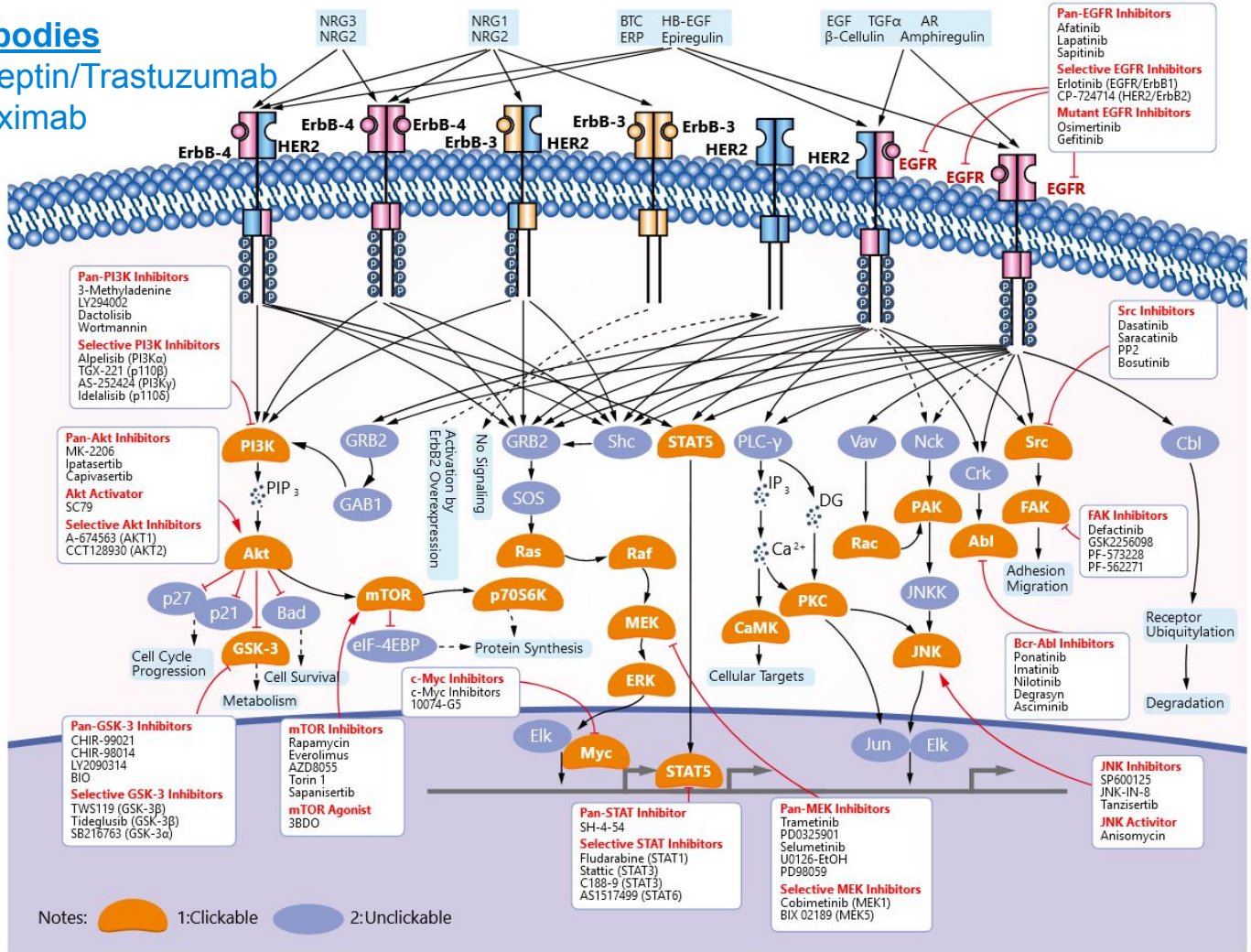
Criteria to choose a modality



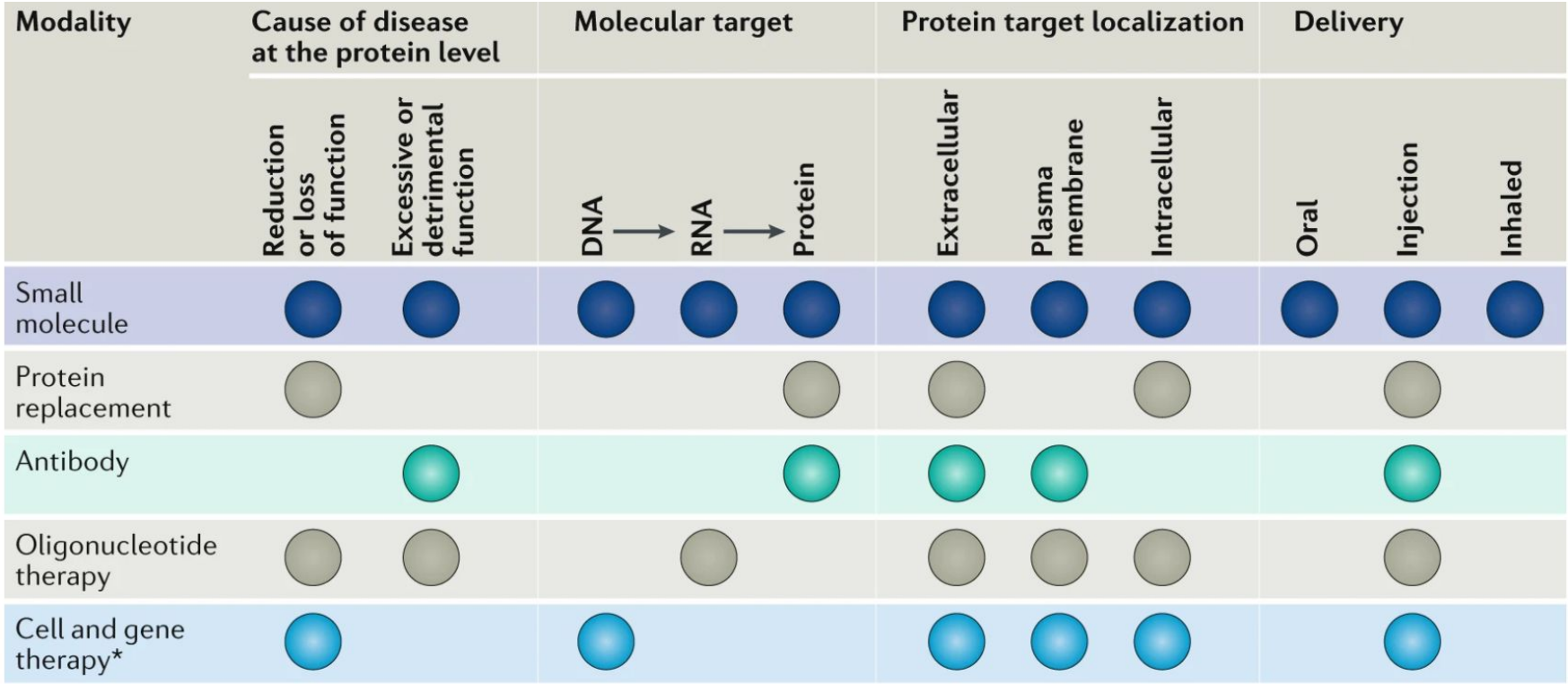
Multiple modalities can target the same biological process

An example: the epidermal growth factor receptor (EGFR) pathway

Antibodies
Herceptin/Trastuzumab
Cetuximab



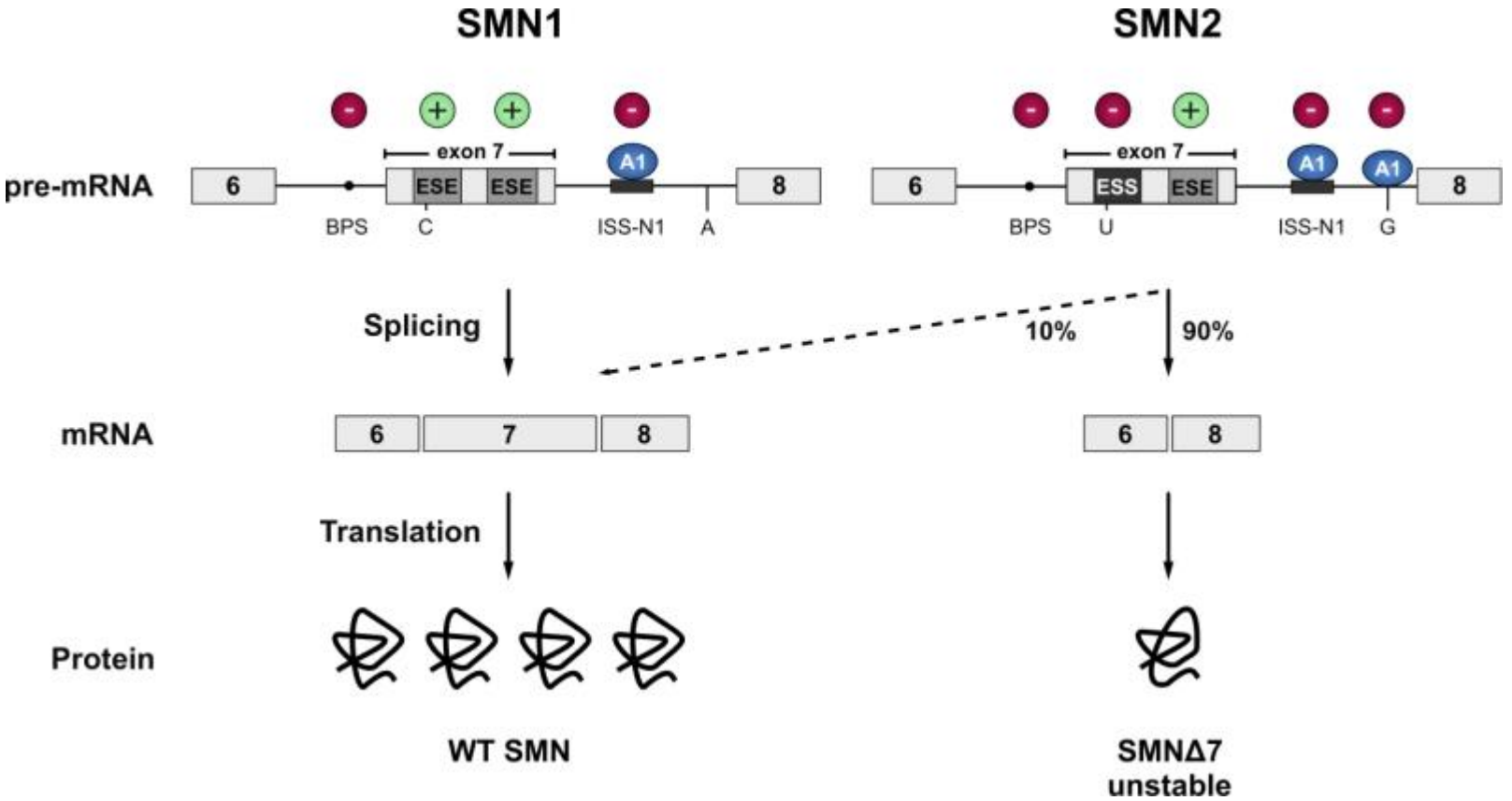
Characteristics of therapeutic modalities



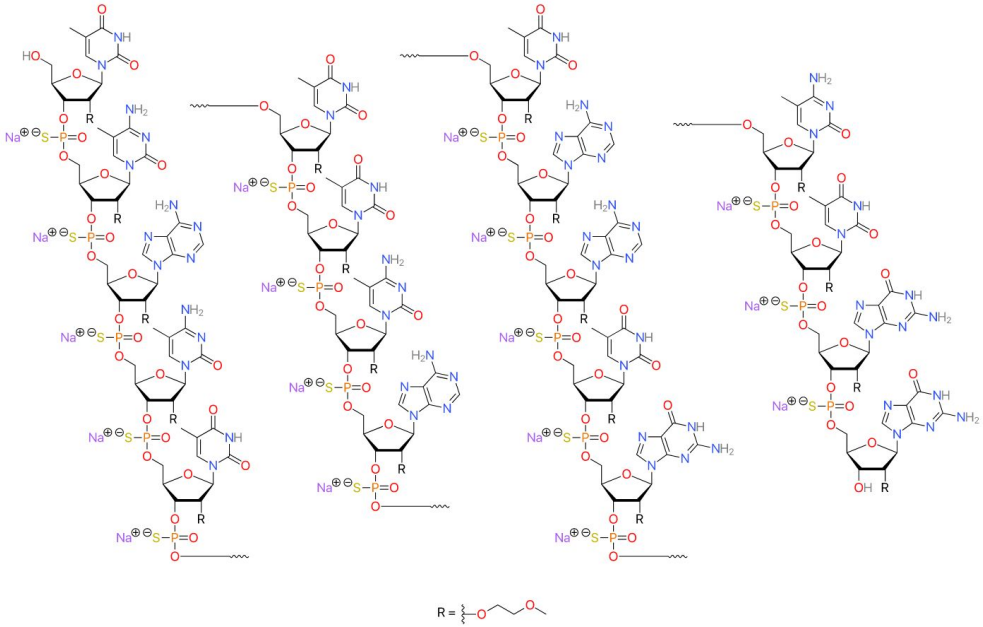
Facts about Spinal Muscular Atrophy (SMA)

- SMA is caused by a defect in a gene called *SMN1*. People with SMA have reduced levels of the SMN protein.
- When SMN protein levels are reduced, motor neurons are unable to send signals to the muscles, causing them to become smaller and weaker over time
- Depending on the severity, or type of SMA, people with the disease will have difficulties moving, eating, and in some cases breathing, making them increasingly dependent on parents and caregivers
- A short movie: https://www.nejm.org/doi/full/10.1056/NEJMoa2009965

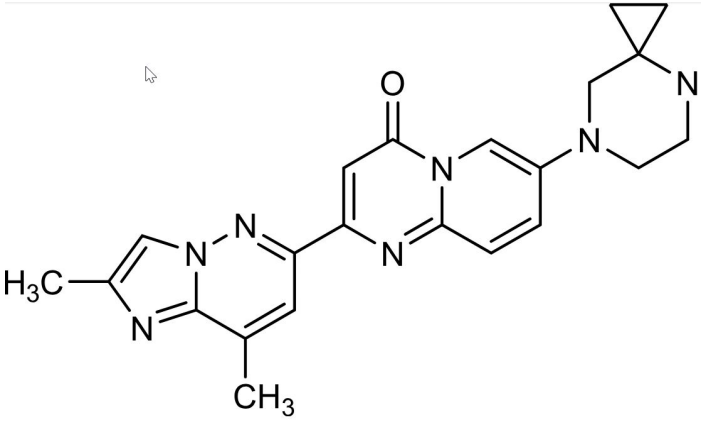
The molecular mechanism of SMA



Two Drugs, One Disease

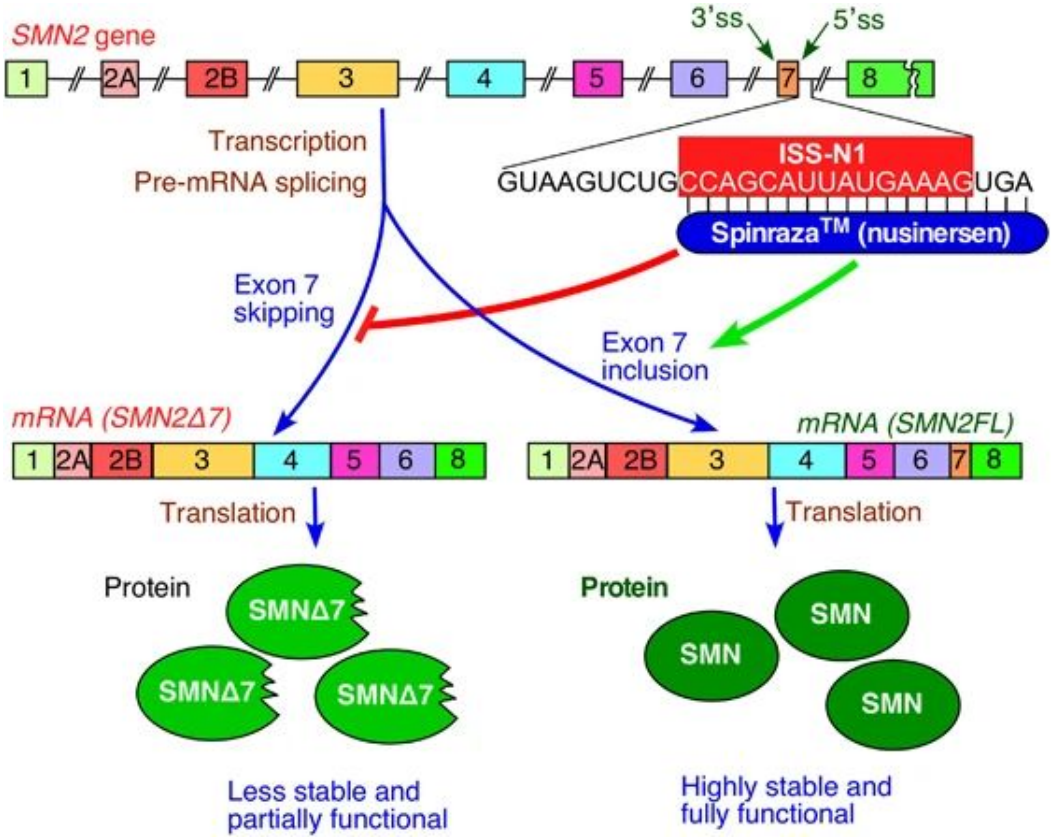


Nusinersen sodium/ Spinraza (CHEMBL3833342)



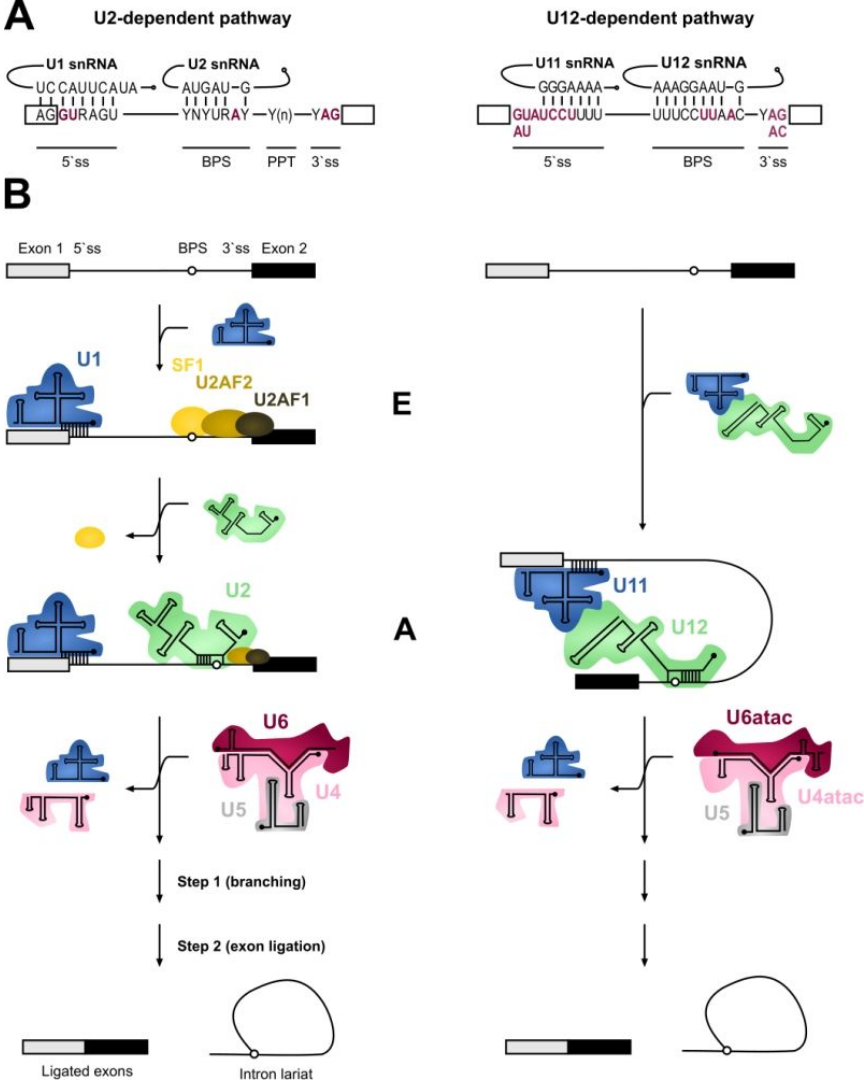
Risdiplam/ *Evrysdi* (CHEMBL4297528)

How Spinaraza (nusinersen) works

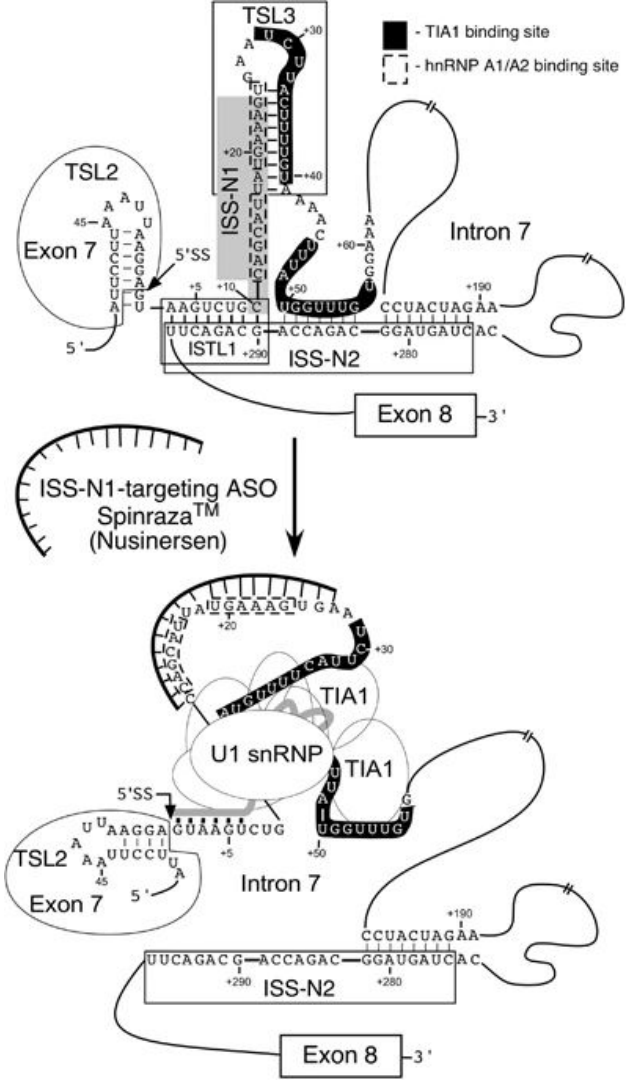


The splicing machinery

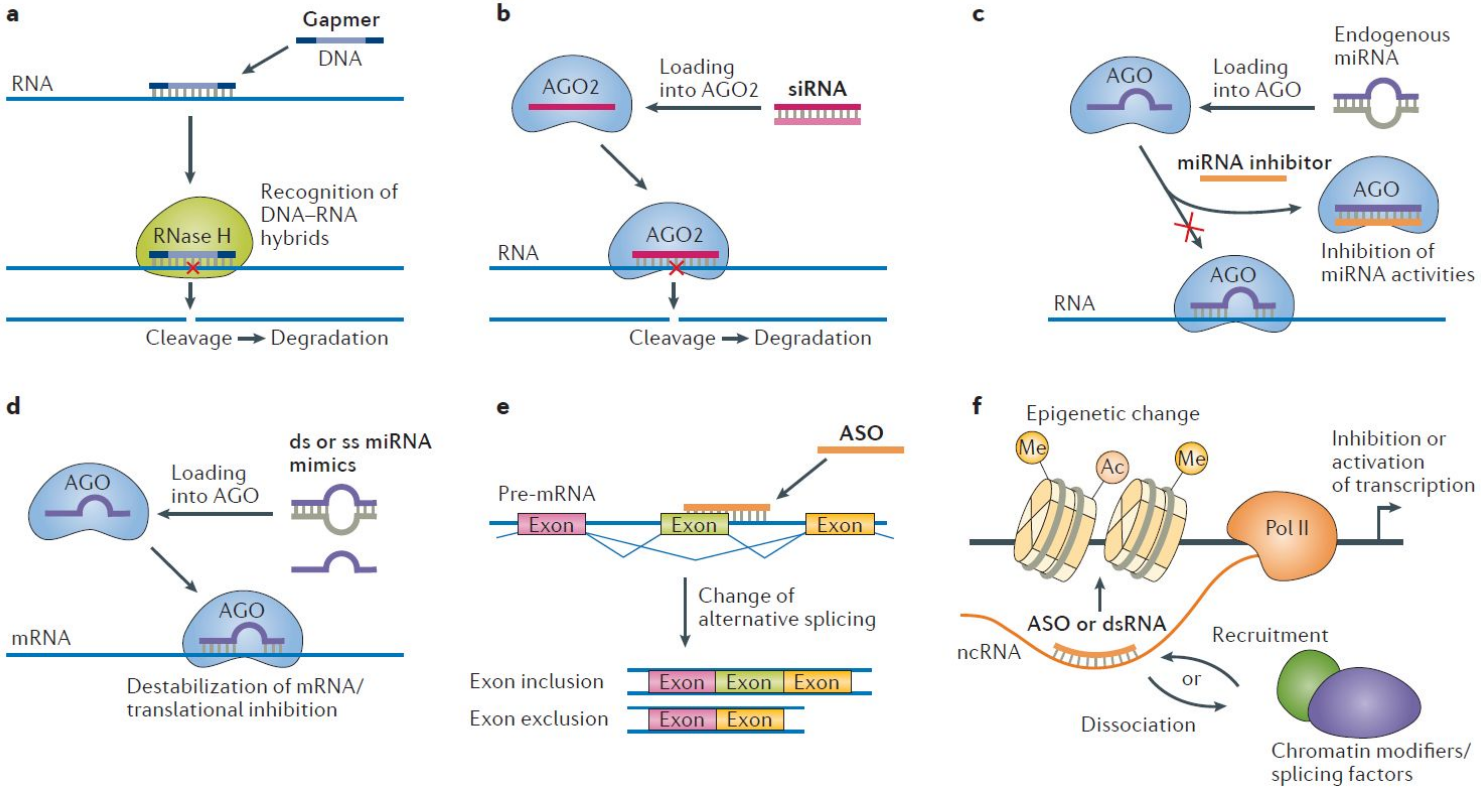
Spliceosome	snRNAs	Core associated proteins
Major	U1	Sm proteins^{+}, U1-A , U1-C, U1-70K
	U2	Sm proteins^{+}, **12S**$^{\#}$: U2-A', U2-B'', 17S$^{\#}$: SF3a and SF3b complexes, hPrp43
	U5*	Sm proteins^{+}, **20S**$^{\#}$: 52K, 40K, hPrp8, hBrr2, Snu114, hPrp6, hPrp28, hDib1
	U4/U6	Sm proteins^{+}, LSm proteins2-8, **13S**$^{\#}$: CypH, 15.5K, hPrp3, hPrp31, hPrp4
Minor	U11/U12	Sm proteins^{+}, **18S**$^{\#}$: SF3b complex, 20K (*ZMAT5*), 25K (*SNRNP25*),31K (*ZCRB1*) 35K (*SNRNP35*), 48K (*SNRNP48*), 59K (*PDCD7*) 65K (*RNPC3*),Urp (*ZRSR2*)
	U4atac/U6atac	Share proteins with U4/U6 snRNAs of the major spliceosome



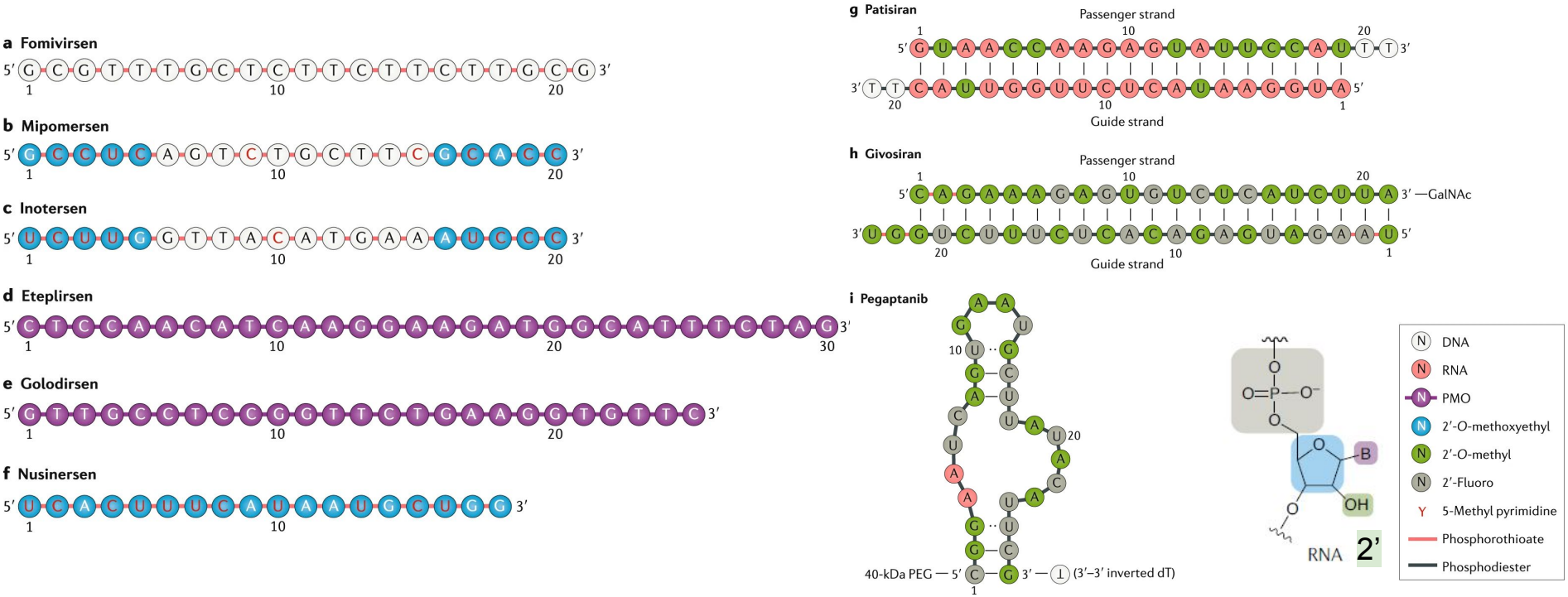
How Spinaraza (nusinersen) works, base by base



Regulating RNA levels or splicing with ASOs and duplex RNAs

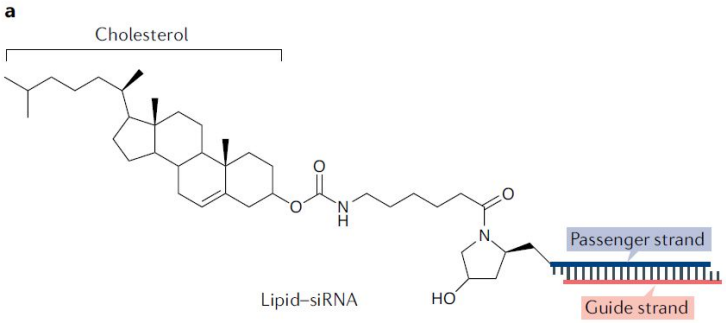


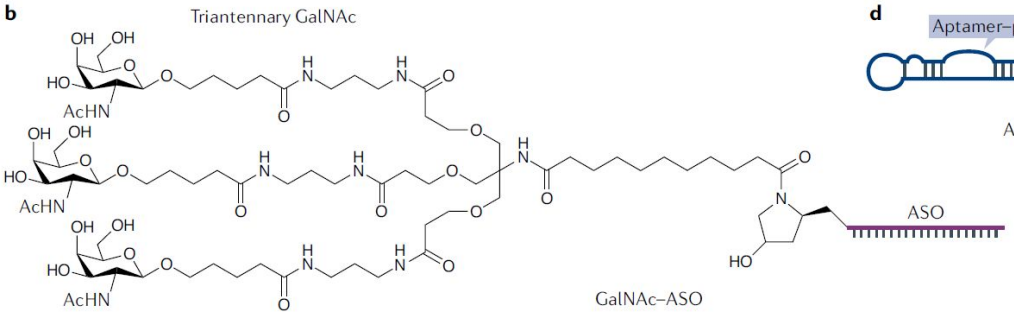
Chemistry of oligonucleotides evolves with time

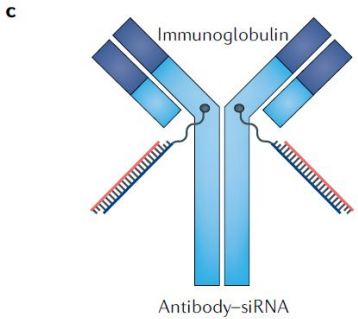


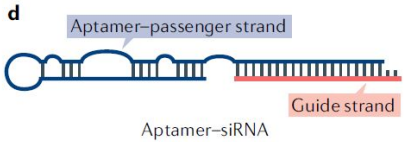
PMO=phosphorodiamidate morpholino oligomer

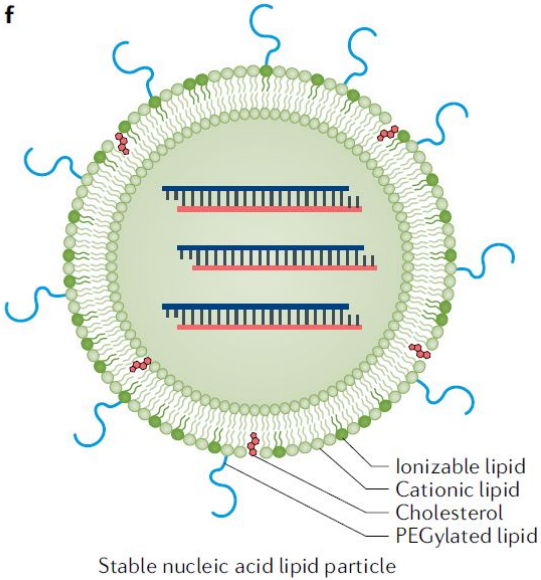
Delivery systems of antisense oligonucleotides



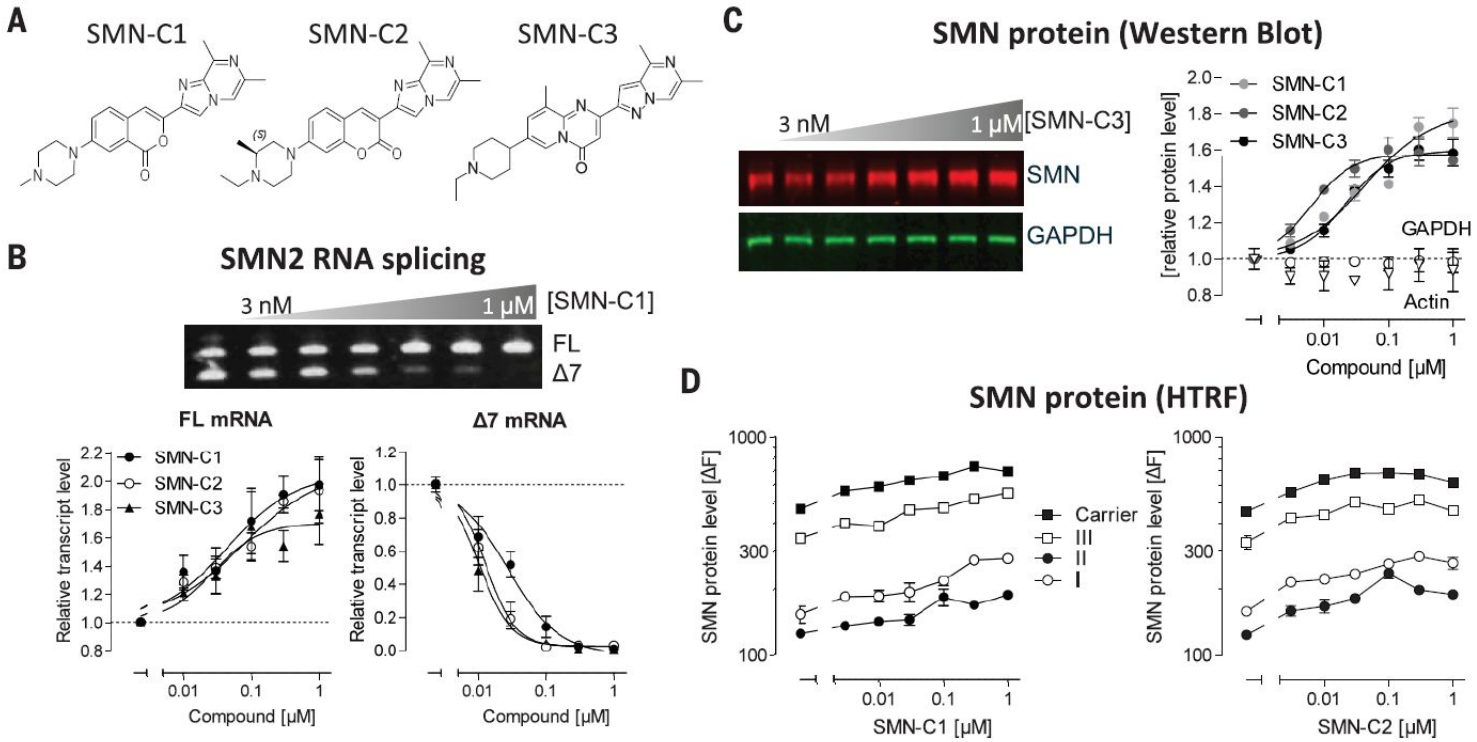




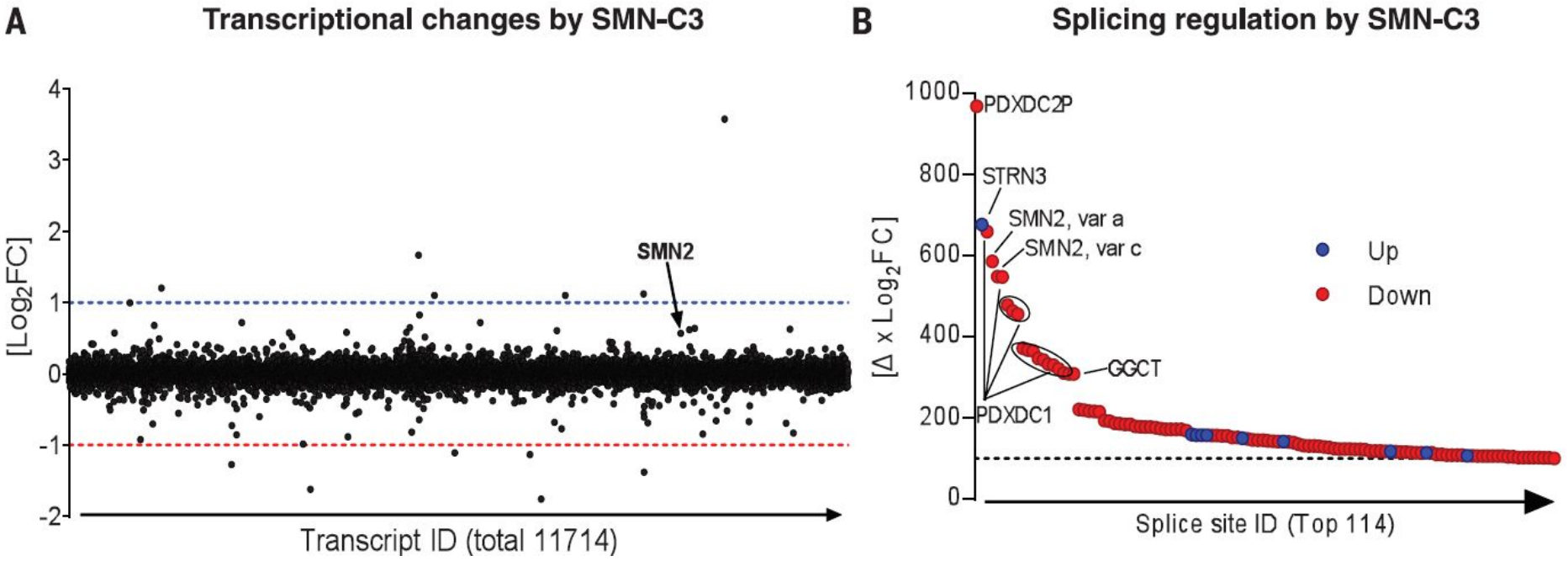




Small molecules as RNA splicing modifiers

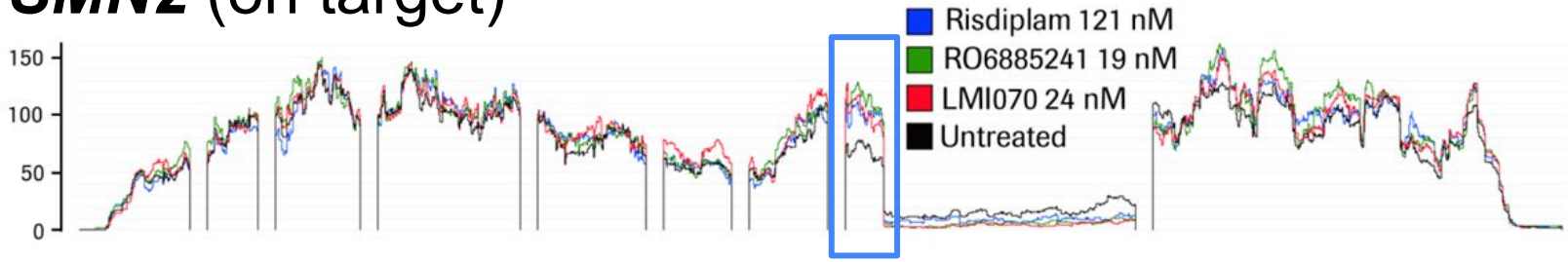


RNA sequencing confirms the specificity of SMN-C3

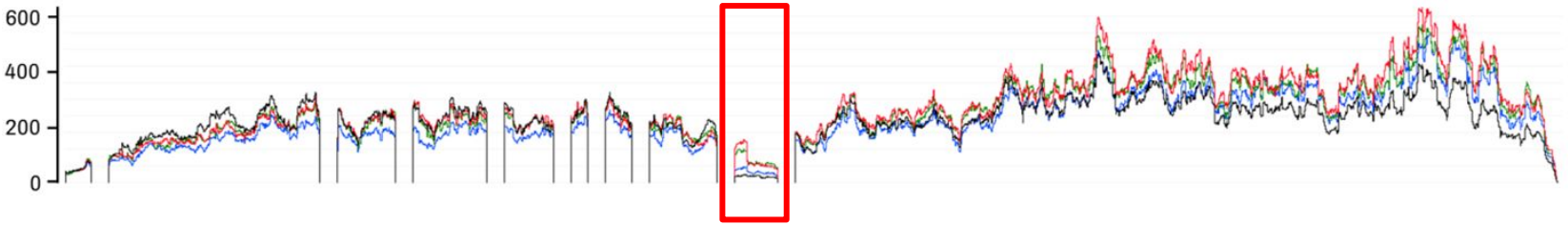


RNA sequencing confirms the specificity of SMN-C3

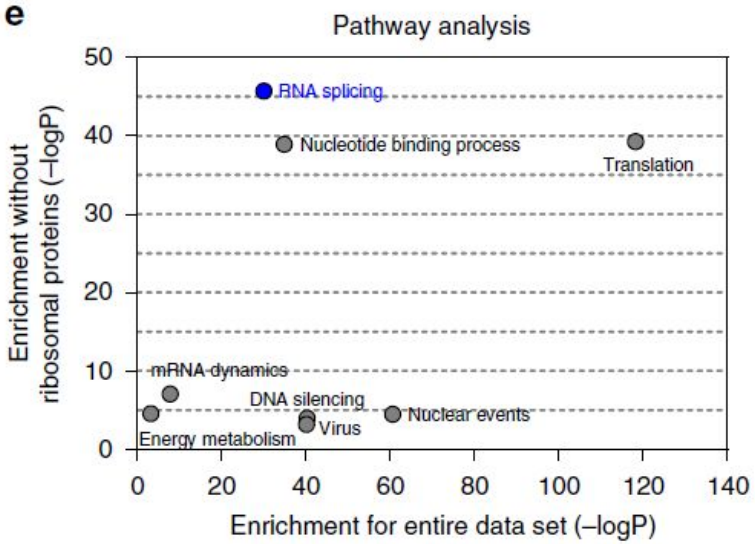
SMN2 (on target)



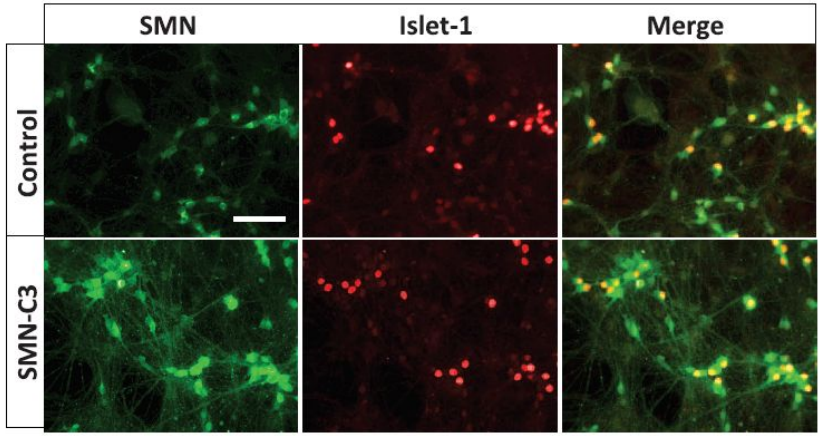
FOXM2 (off target)

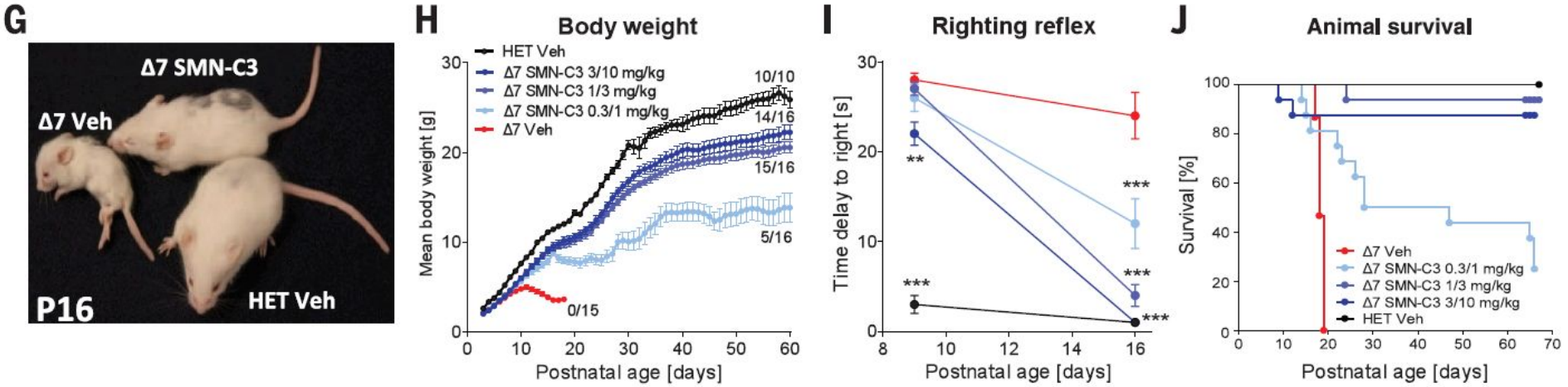


Gene-enrichment analysis confirms specific regulation of RNA splicing

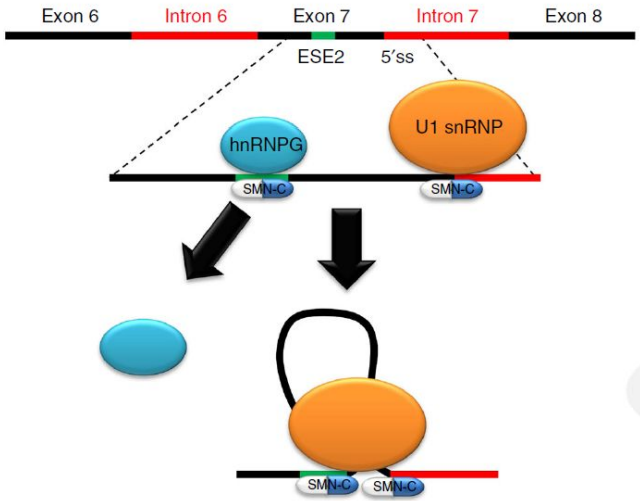


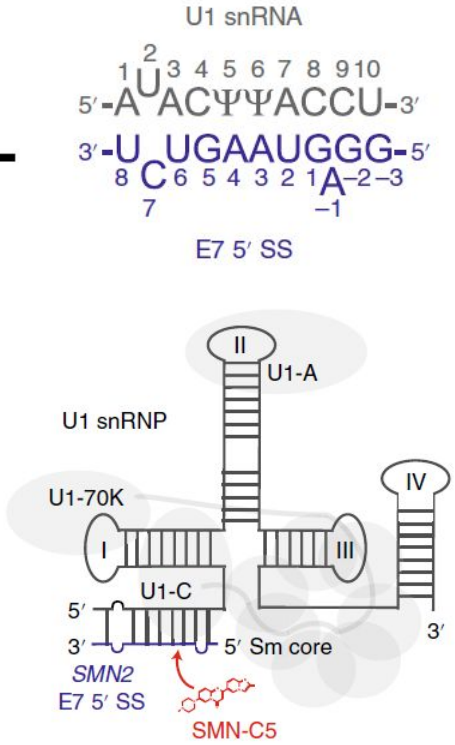
Experiments *in vitro* and *in vivo* support efficacy profiles of SMN-C3

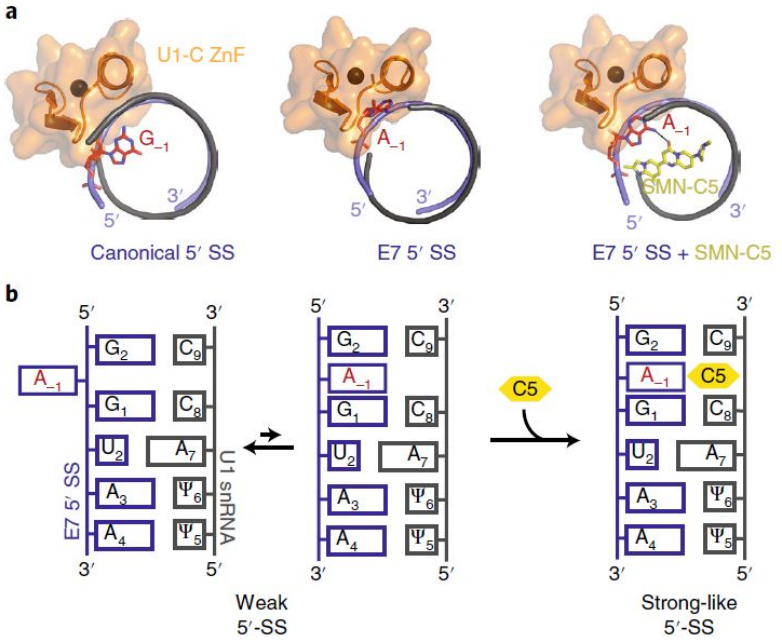




Structural basis of specific splicing correction







FIREFISH Part 1 Results

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

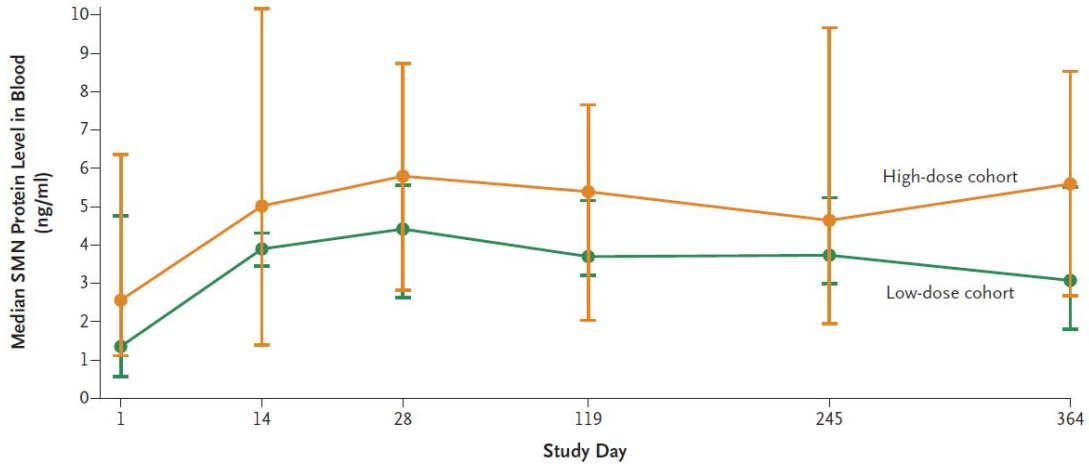
Characteristic	Low-Dose Cohort (N=4)	High-Dose Cohort (N=17)	All Infants (N=21)
Sex — no. (%)			
Female	4 (100)	11 (65)	15 (71)
Male	0	6 (35)	6 (29)
Median age (range) — mo			
At onset of symptoms	2.7 (2.0–3.0)	1.5 (0.9–3.0)	2.0 (0.9–3.0)
At diagnosis	3.3 (2.5–5.1)	3.0 (0.9–5.4)	3.0 (0.9–5.4)
At enrollment	6.9 (6.7–6.9)	6.3 (3.3–6.9)	6.7 (3.3–6.9)
Motor measures†			
Median CHOP-INTEND score (range)	23.5 (10–25)	24 (16–34)	24 (10–34)
Median HINE-2 score (range)	1 (0–3)	1 (0–2)	1 (0–3)
Respiratory support — no. (%)	0	5 (29)‡	5 (24)‡

Note: Table 2 is not complete

Table 2. Adverse Events.*

Event	Infants (N=21)
Total no. of adverse events	202
≥1 Adverse event — no. (%)	21 (100)
Total no. of serious adverse events	24
≥1 Serious adverse event — no. (%)	10 (48)
≥1 Adverse event of grade 3–5 — no. (%)	9 (43)
Serious adverse event with fatal outcome — no. (%)†	3 (14)
Most common adverse events — no. (%)‡	
Pyrexia	11 (52)
Upper respiratory tract infection	9 (43)
Diarrhea	6 (29)
Cough	5 (24)

FIREFISH Part 1 Results



No. of Patients						
High-dose cohort	16	13	17	15	15	15
Low-dose cohort	4	3	3	3	3	3

Median SMN Protein Level in Blood (range) — ng/ml						
High-dose cohort	2.54 (1.10–6.40)	5.01 (1.42–10.22)	5.87 (2.84–8.76)	5.44 (2.03–7.71)	4.64 (1.95–9.67)	5.66 (2.66–8.60)
Low-dose cohort	1.31 (0.58–4.82)	3.87 (3.45–4.37)	4.49 (2.61–5.55)	3.67 (3.24–5.21)	3.71 (2.97–5.24)	3.05 (1.75–5.51)

Figure 1. SMN Protein Concentration in Whole Blood.

Blood was mixed with lysis buffer in a 1:1 ratio. I bars indicate the range. The data-cutoff date was February 27, 2019. SMN denotes survival of motor neuron.

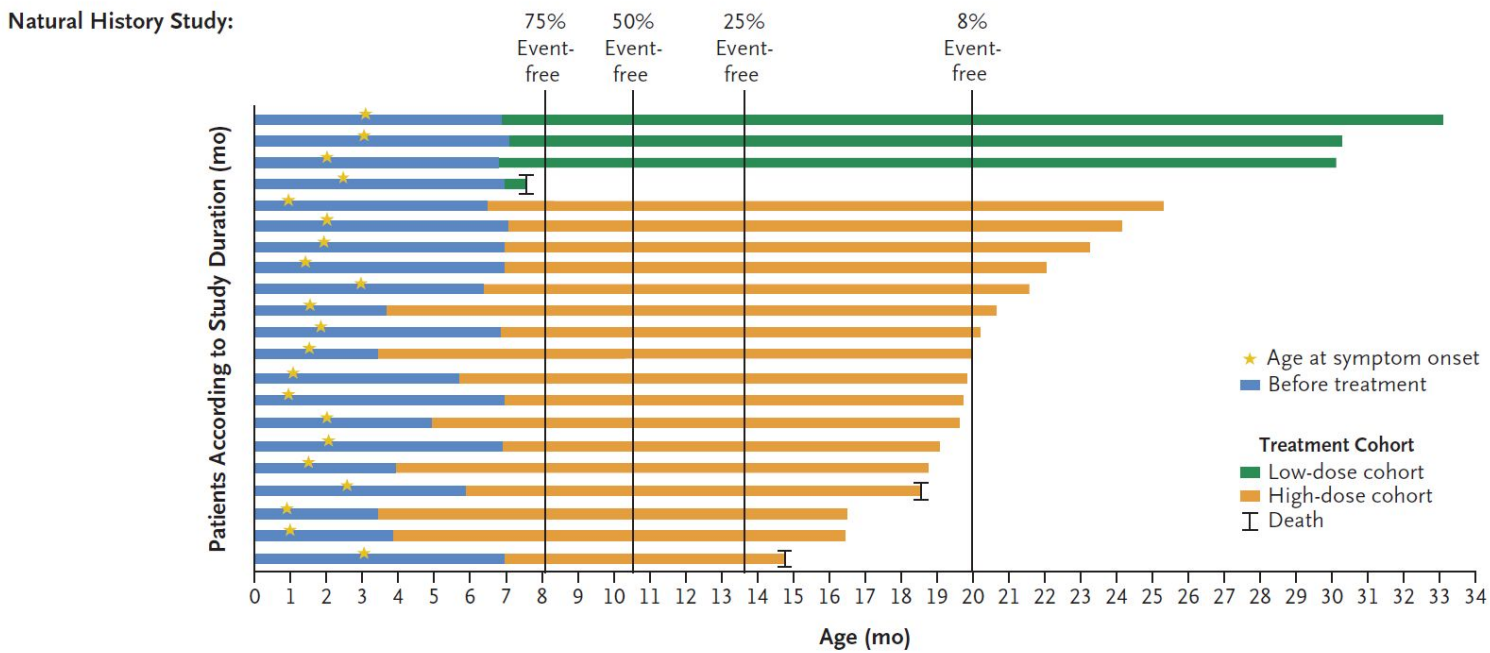


Figure 2. Event-free Survival.

Event-free survival was defined as being alive and not receiving permanent ventilation (tracheostomy or ventilation [bilevel positive airway pressure] for ≥16 hours per day continuously for >3 weeks or continuous intubation for >3 weeks, in the absence of, or after the resolution of, an acute reversible event). The percentages of patients who were event-free in a previous natural history study of spinal muscular atropy[7] are shown at the top of the graph for comparison. The median age at the combined outcome among patients in the previous study who had two copies of *SMN2* was 10.5 months (interquartile range, 8.1 to 13.6); event-free survival in that study was defined as being alive and not receiving noninvasive ventilation for 16 hours or more per day continuously for 2 or more weeks. The duration of our study was measured from the date of enrollment to the data-cutoff date. As of the data-cutoff date, three infants (one in the low-dose cohort and two in the high-dose cohort) had died; one additional infant in the high-dose cohort died after that date (Table S5).

Enzymic and genetic inhibition have distinct impact on reaction dynamics

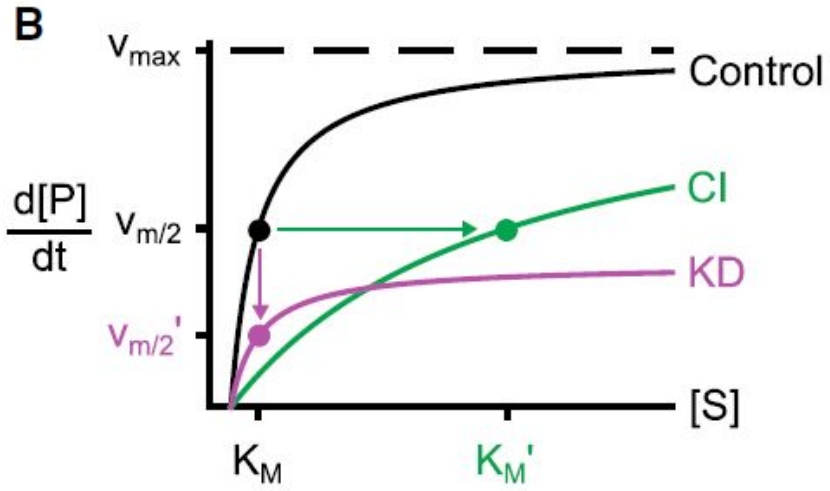
A

$$[E] + [S] \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E\text{-}S] \xrightarrow{k_{cat}} [E] + [P]$$

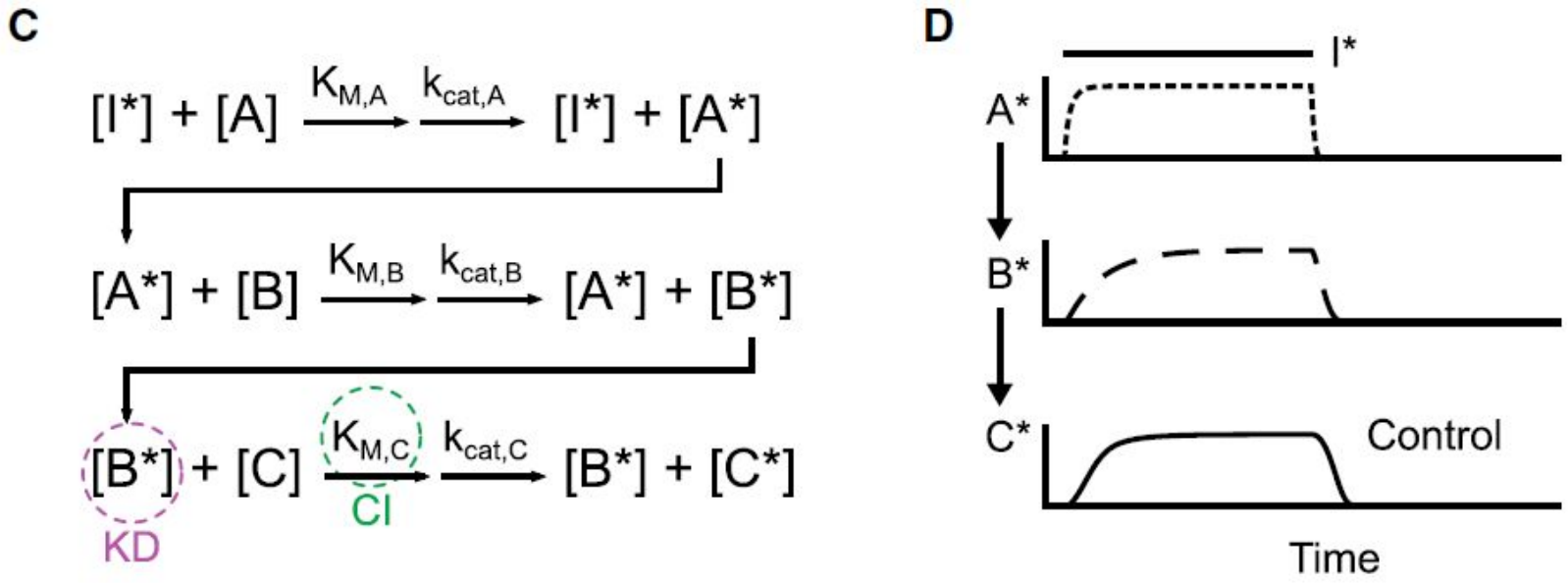
$$\frac{d[P]}{dt} = v_{max}\frac{[S]}{[S] + K_M}$$

$$K_M = \frac{k_{-1} + k_{cat}}{k_1} \qquad v_{max} = k_{cat}[E]_o$$

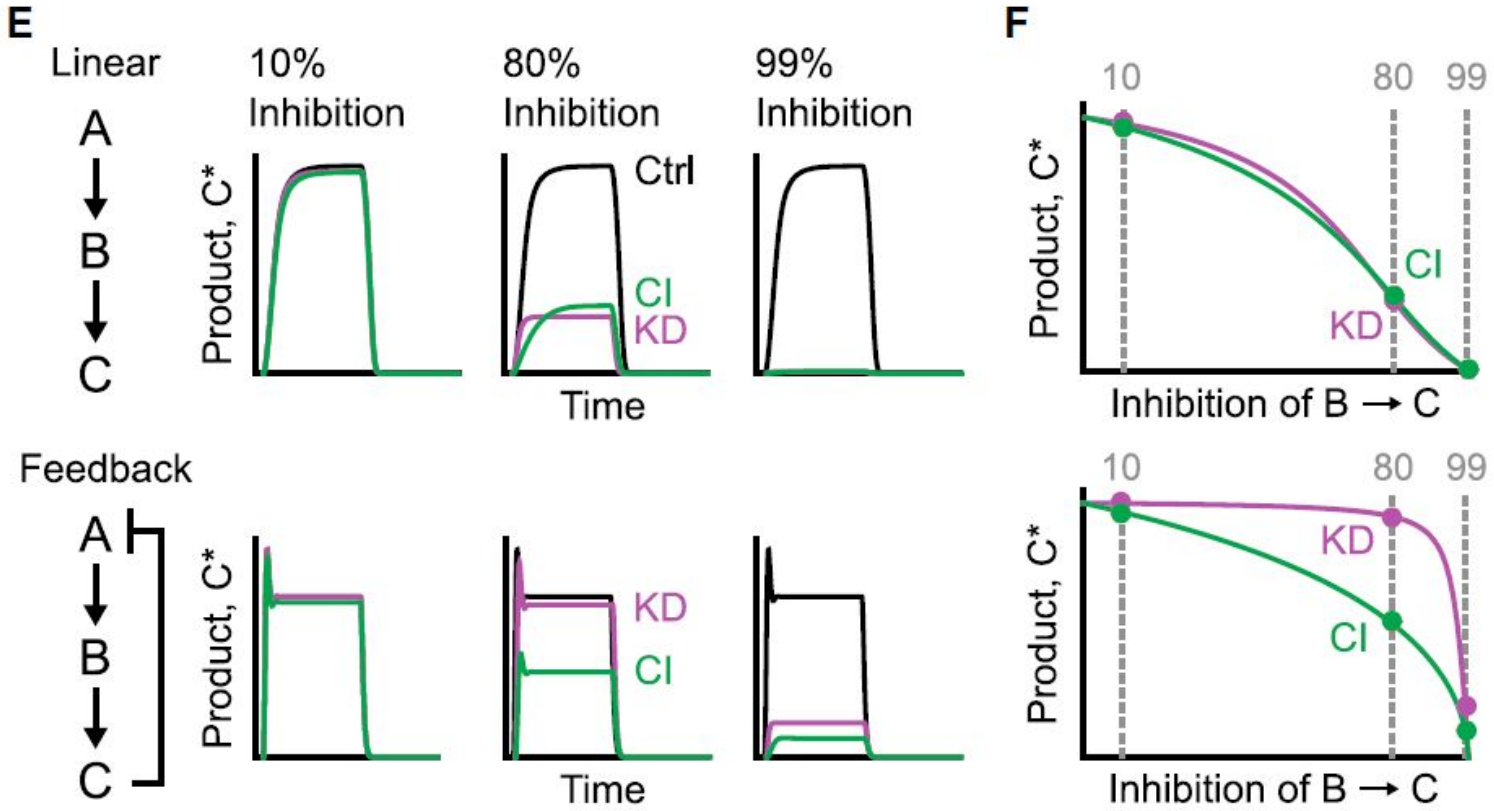
B



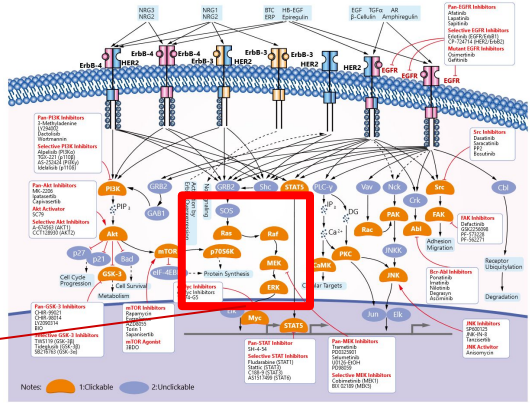
A linear system of enzymatic reactions

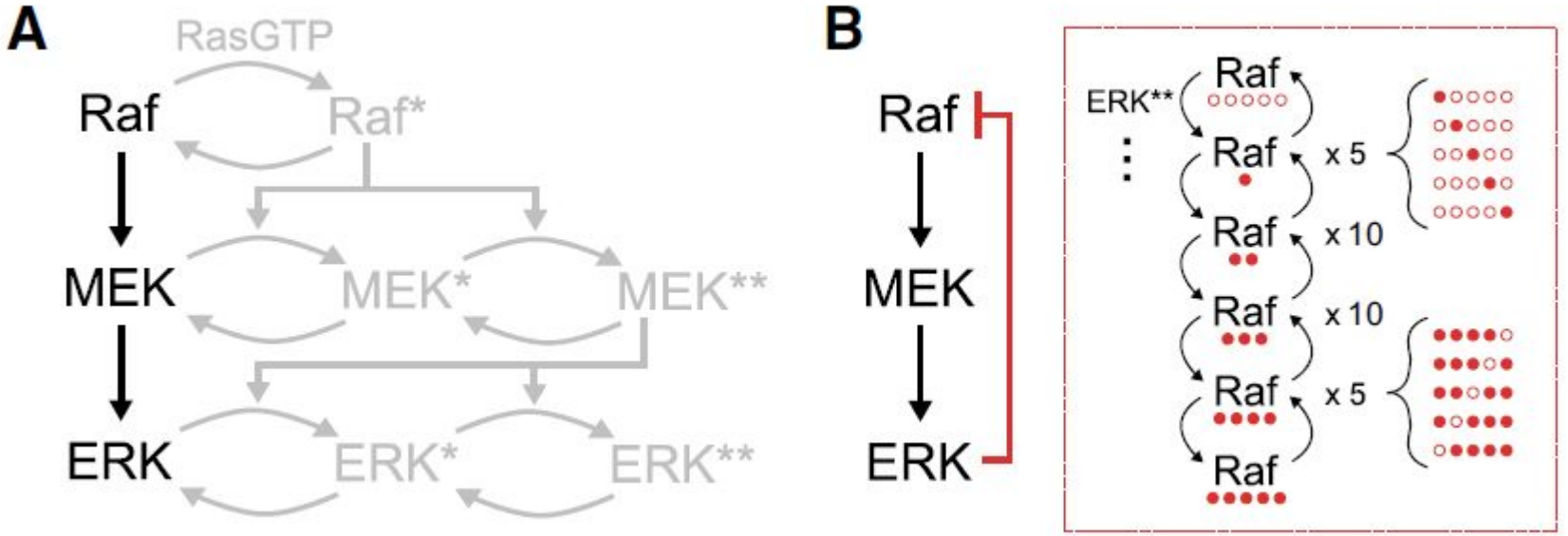


Network architecture may differentiate effects of enzymatic and genetic inhibition

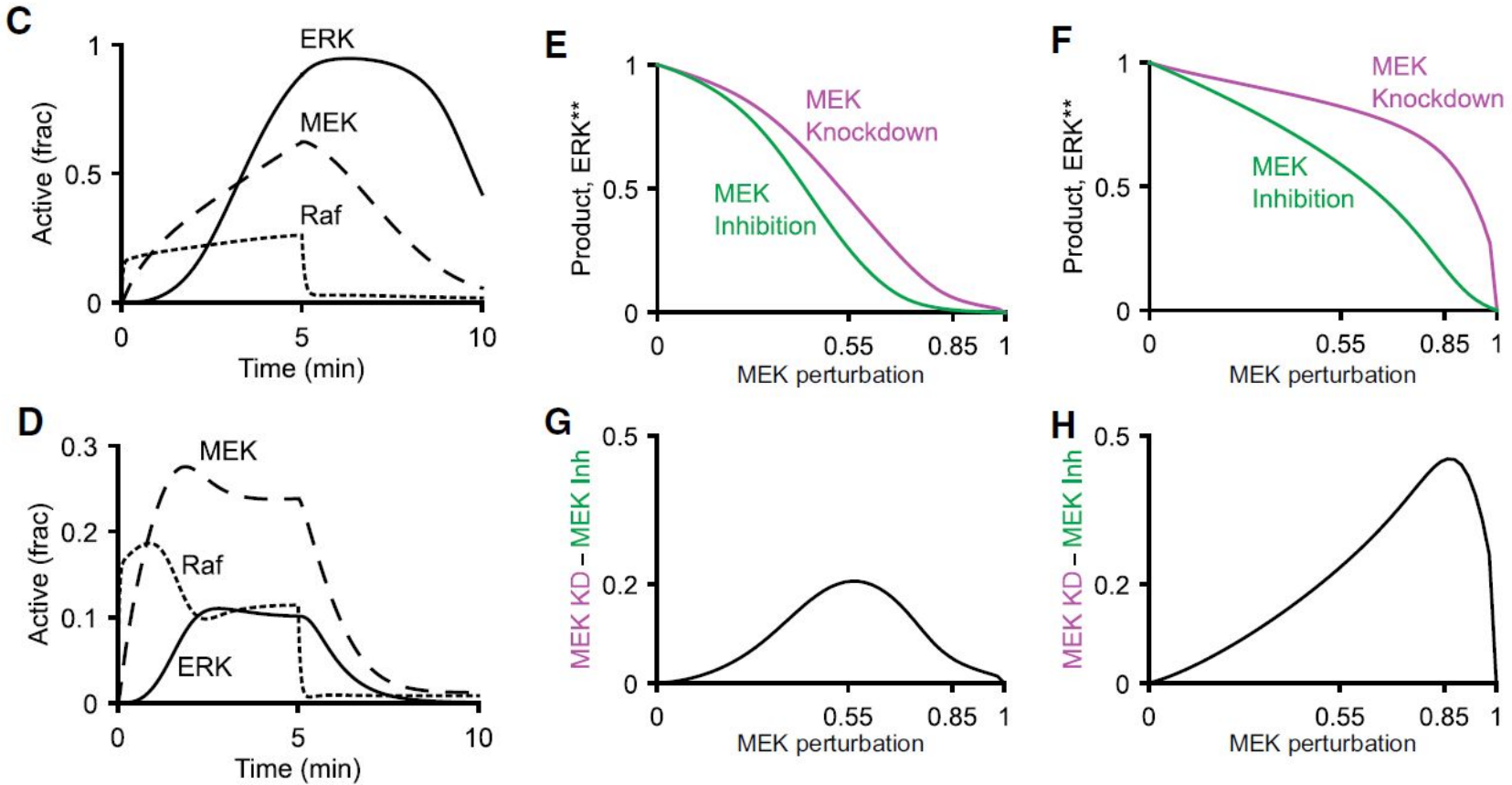


The MAPK/ERK pathway downstream of EGFR signalling

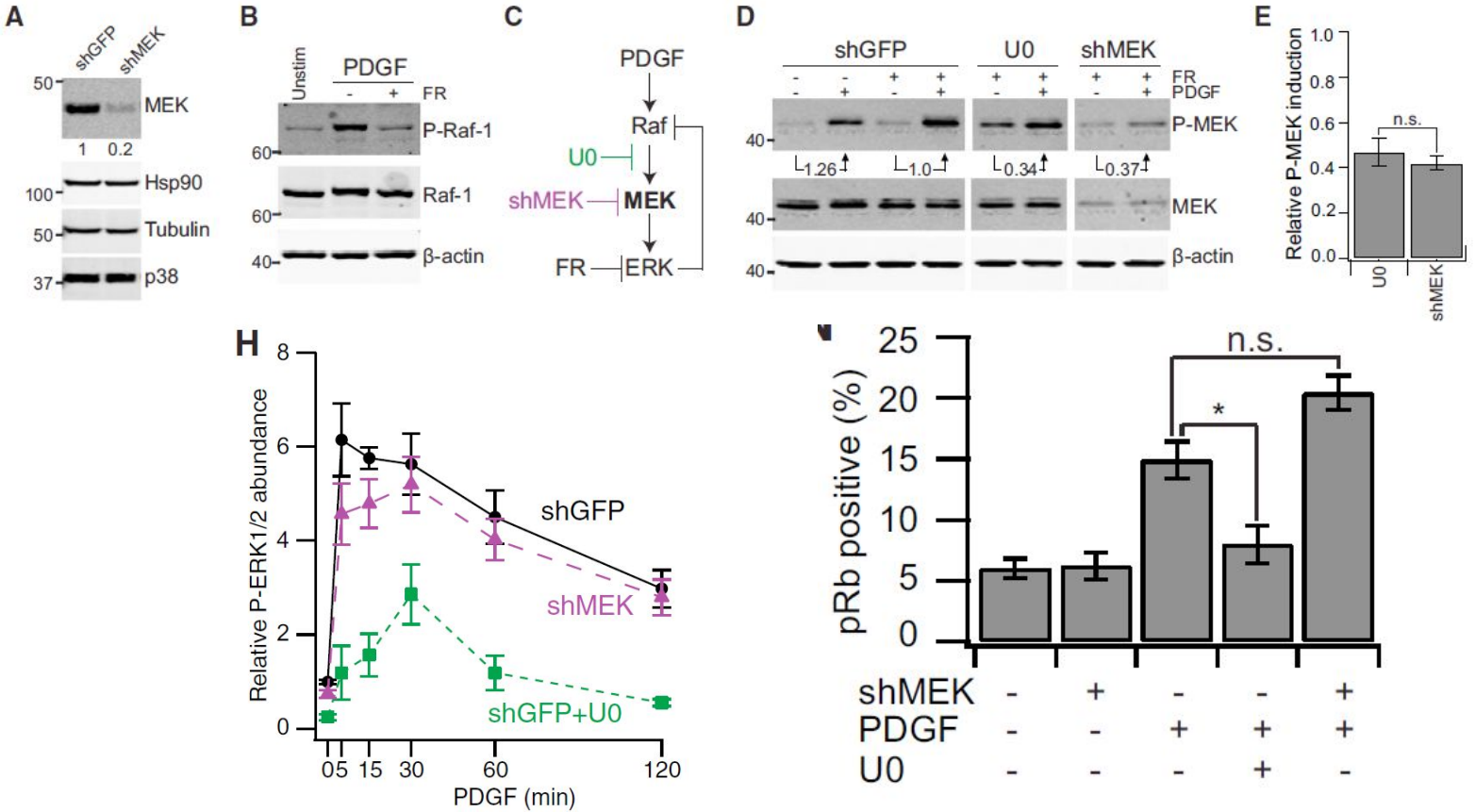




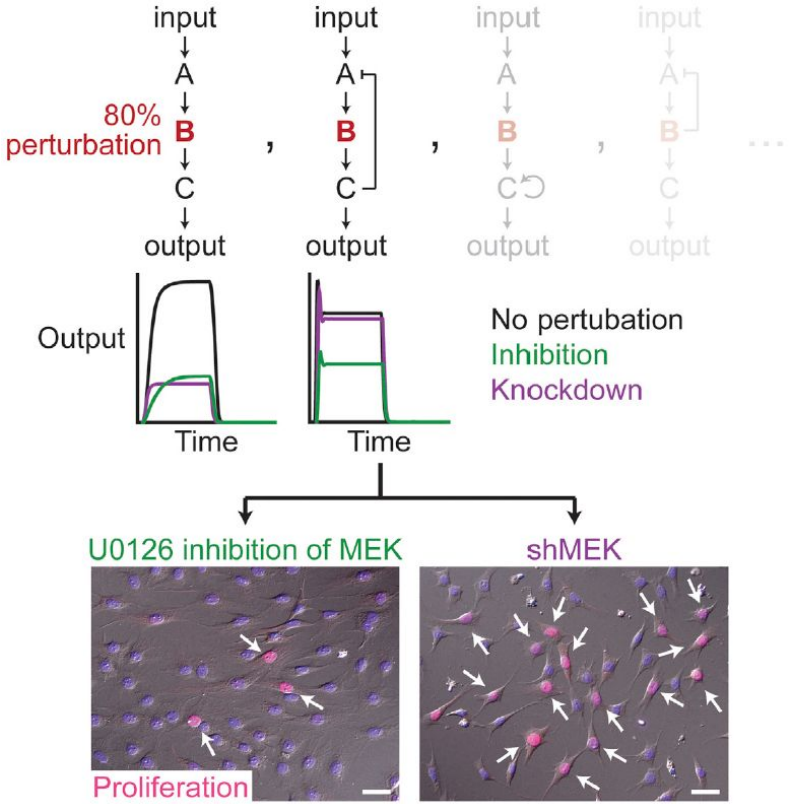
A model predicts different effect of KD and CI



Experimental data is in line with model prediction

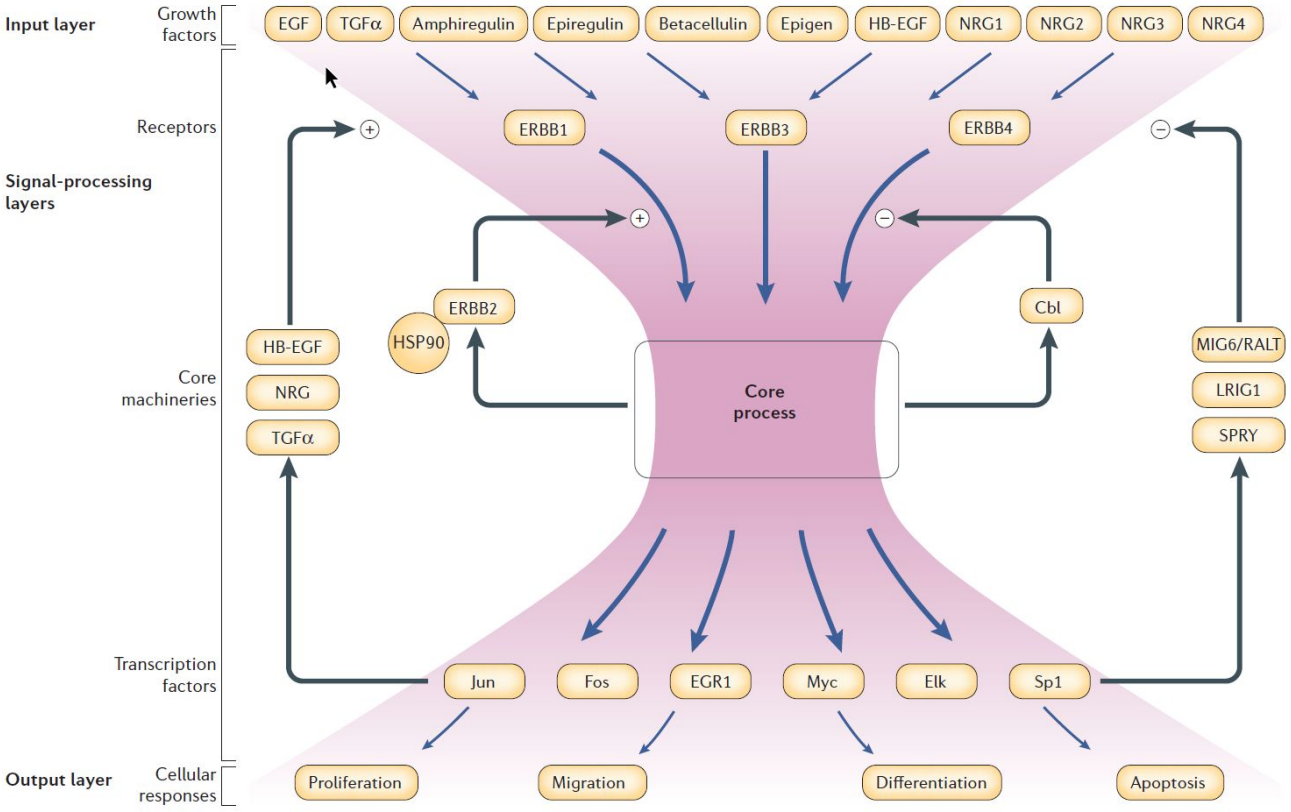


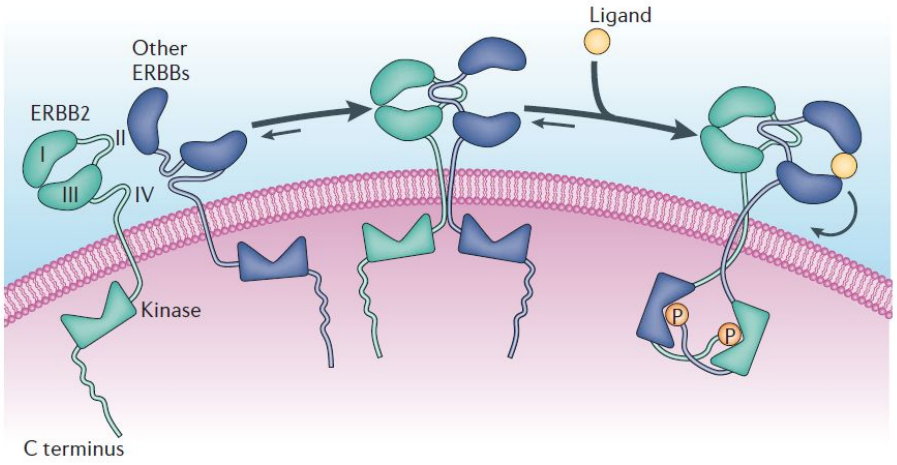
Network architecture around an enzyme can affect our choice of modality

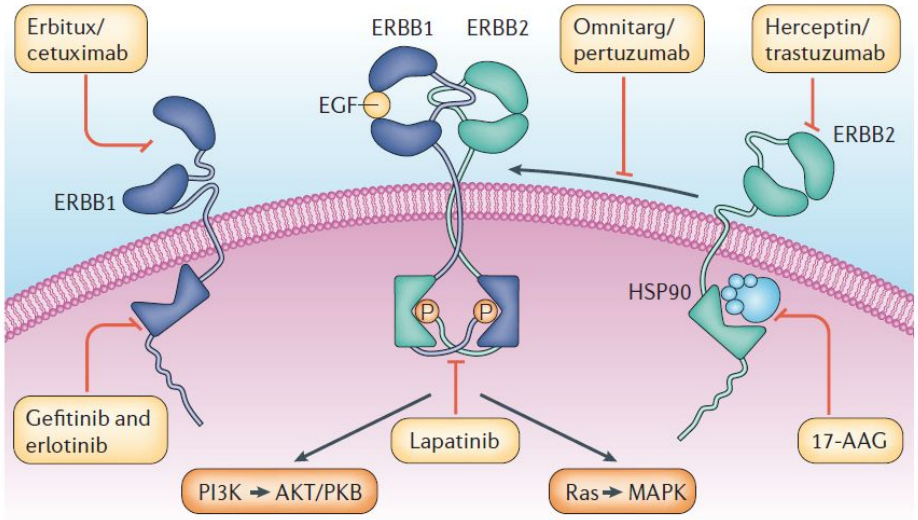


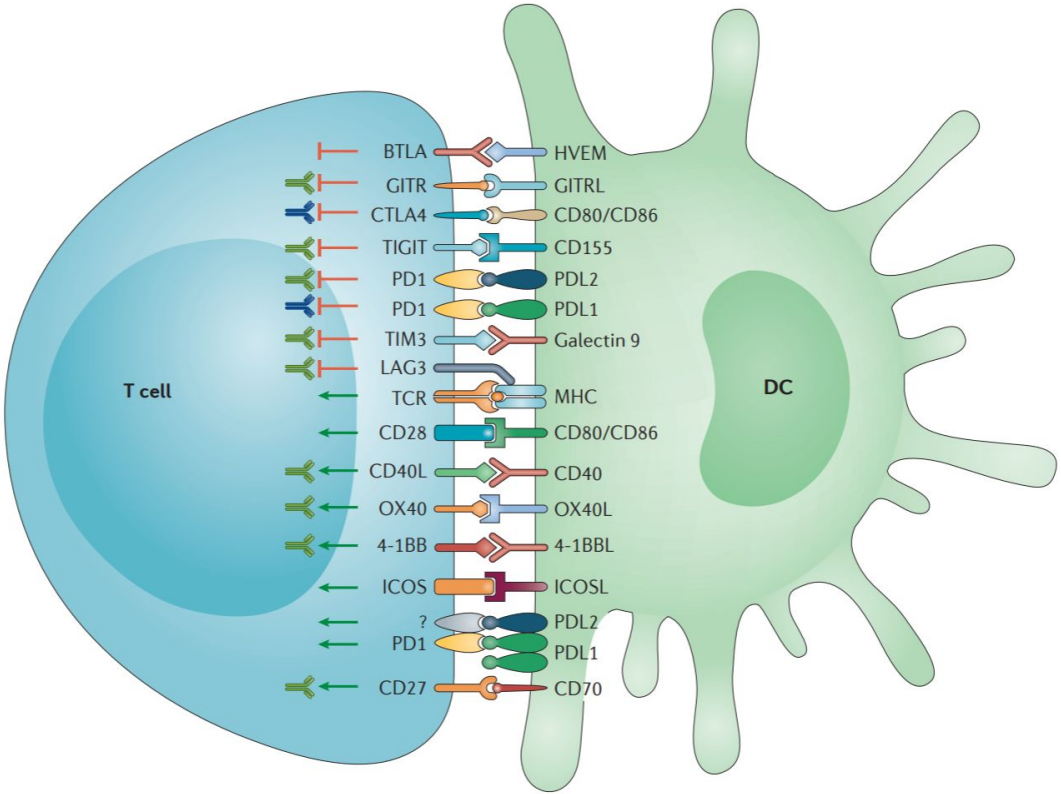
Conclusions

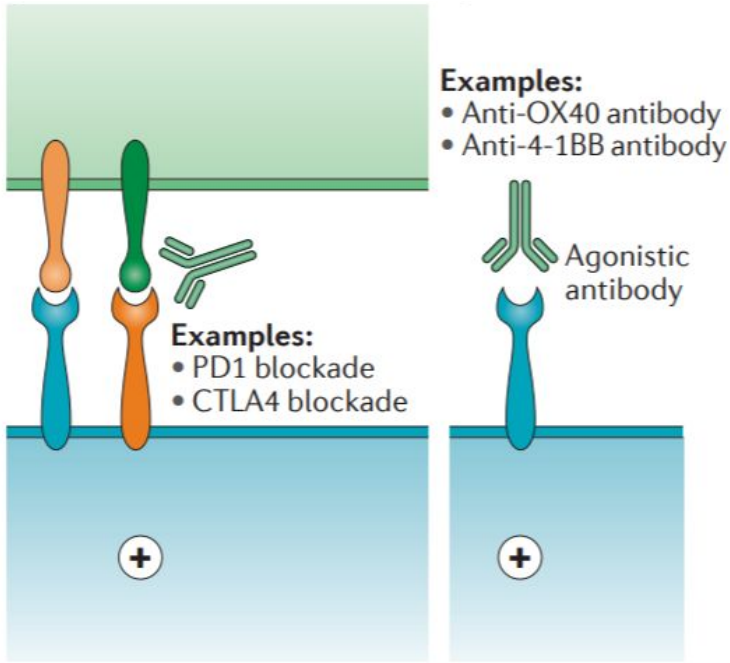
- Given mechanistic understanding of biological processes underlying diseases, we can develop different modalities as therapeutics;
- Mathematical and computational biology (1) helps with molecule design, (2) reveals how drug candidate work and ranks them, and (3) contributes to modality selection;


Input layer
Growth factors
EGF
TGFα
Amphiregulin
Epiregulin
Betacellulin
Epigen
HB-EGF
NRG1
NRG2
NRG3
NRG4
Receptors
ERBB1
ERBB3
ERBB4
Signal-processing layers
ERBB2
HSP90
Cbl
HB-EGF
NRG
TGFα
Core machineries
Core process
MIG6/RALT
LRIG1
SPRY
Transcription factors
Jun
Fos
EGR1
Myc
Elk
Sp1
Output layer
Cellular responses
Proliferation
Migration
Differentiation
Apoptosis


Ligand
Other
ERBBs
ERBB2
I
II
III
IV
Kinase
C terminus
P
P

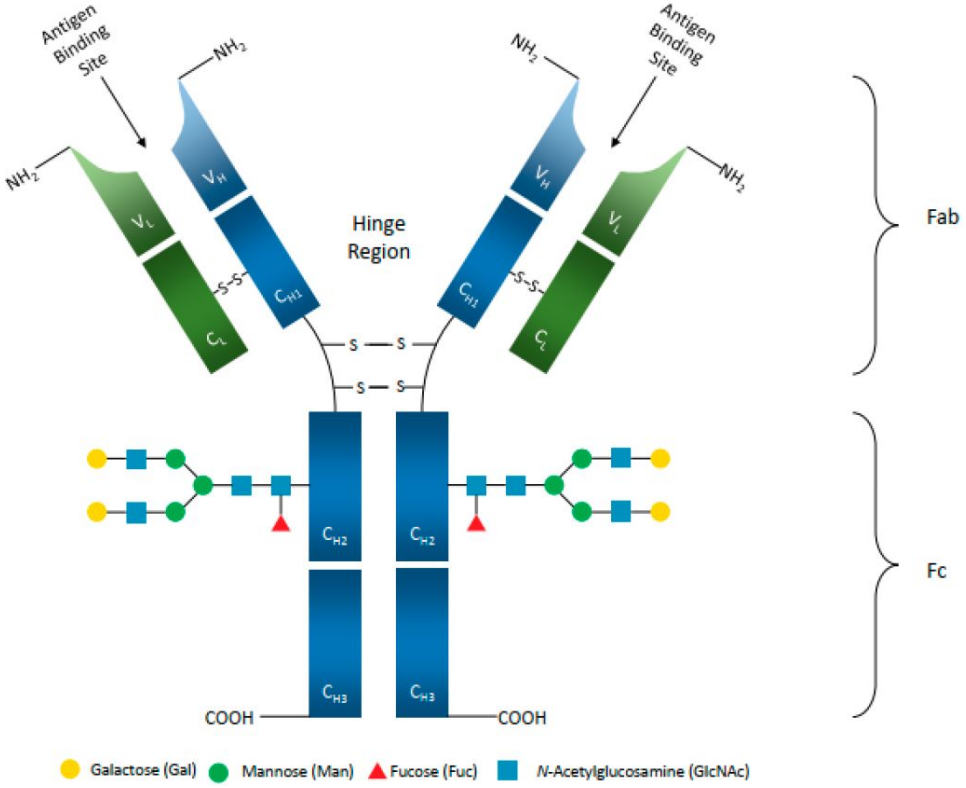

Erbitux/
cetuximab
ERBB1
ERBB1
ERBB2
EGF
Omnitarg/
pertuzumab
Herceptin/
trastuzumab
ERBB2
HSP90
P
P
Gefitinib and
erlotinib
Lapatinib
17-AAG
PI3K → AKT/PKB
Ras → MAPK


T cell
DC
BTLA
HVEM
GITR
GITRL
CTLA4
CD80/CD86
TIGIT
CD155
PD1
PDL2
PD1
PDL1
TIM3
Galectin 9
LAG3
TCR
MHC
CD28
CD80/CD86
CD40L
CD40
OX40
OX40L
4-1BB
4-1BBL
ICOS
ICOSL
?
PDL2
PD1
PDL1
CD27
CD70

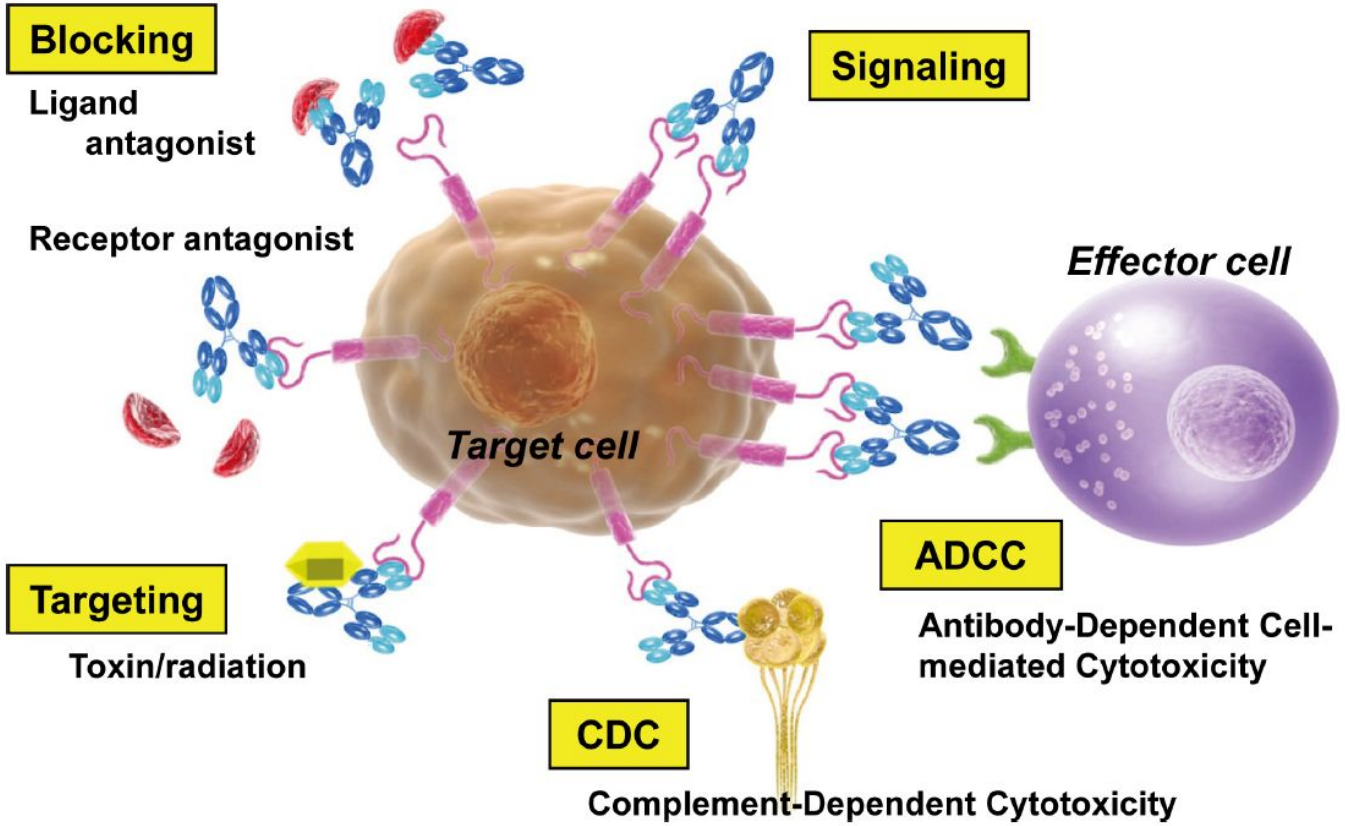

Examples:
• PD1 blockade
• CTLA4 blockade
Examples:
• Anti-OX40 antibody
• Anti-4-1BB antibody
Agonistic antibody
+
+

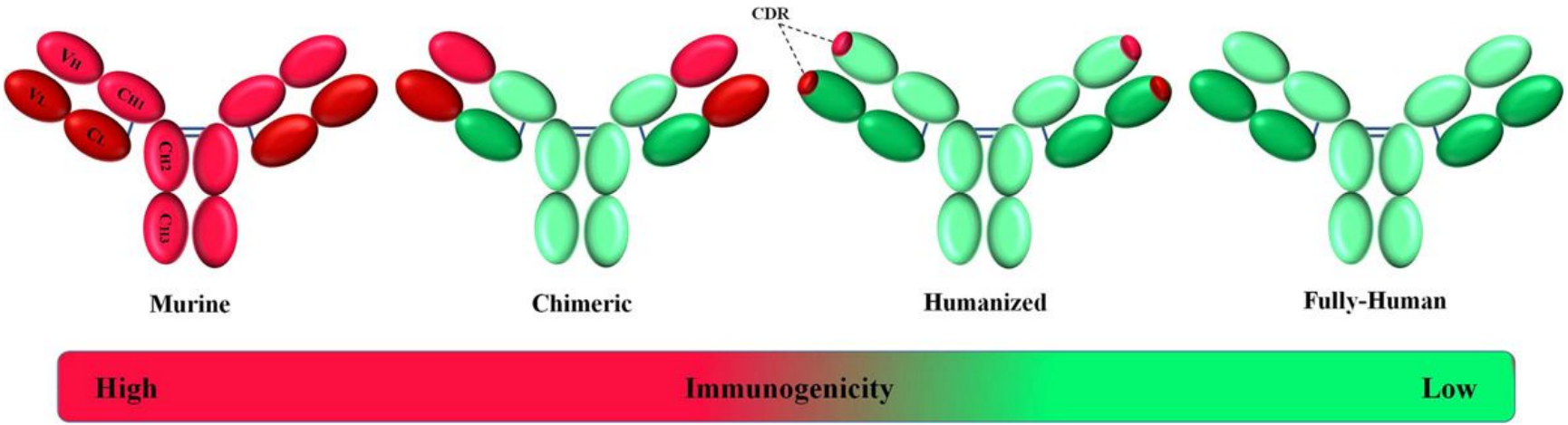
Peptide and antibody

- Antibody design
- Immunogenicity


Antigen Binding Site
NH2
VH
VL
CH1
CL
Hinge Region
S-S
S—S
CH2
CH3
COOH
Fab
Fc
Galactose (Gal)
Mannose (Man)
Fucose (Fuc)
N-Acetylglucosamine (GlcNAc)

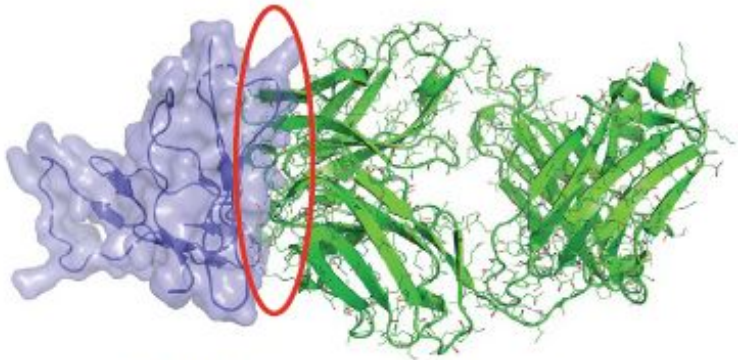
Mechanisms of action of therapeutic antibodies




VH
VL
CH1
CL
CH2
CH3
CDR
Murine
Chimeric
Humanized
Fully-Human
High
Immunogenicity
Low

Multispecific Drug Use or Target Interactions

b Conventional drug:
- Forms 1 drug–target interface
- Can act throughout body
- Only works if its binding to target alters function of target

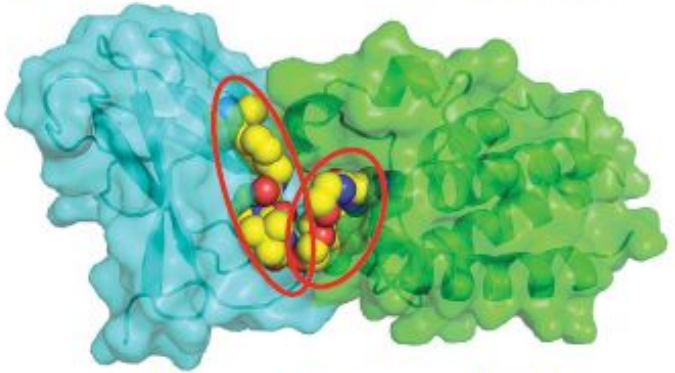


IL-2Rα Basiliximab

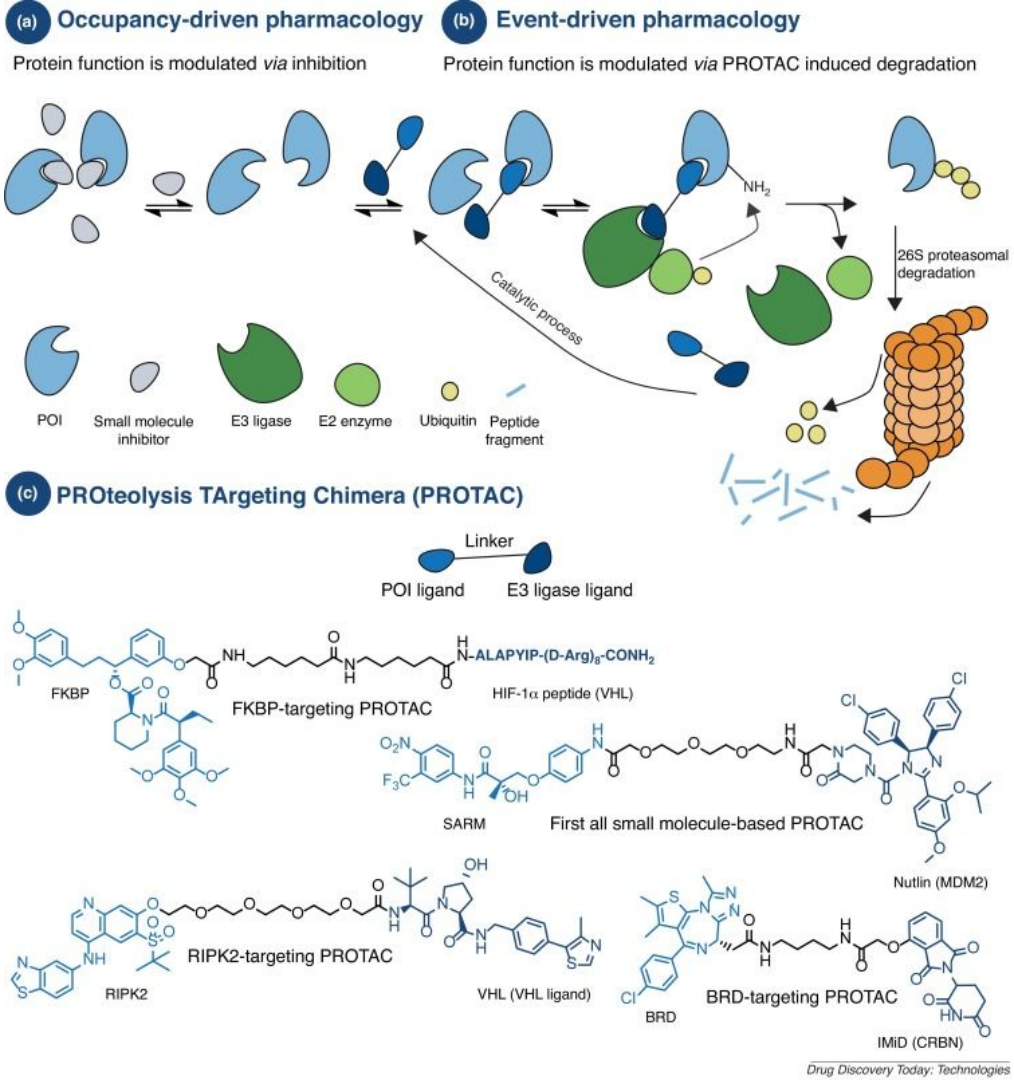
c Obligate multispecific drug:
- Forms 2 or more drug–target interfaces

Class 1 'tetherbodies'
- Enrich drug at relevant site of action

Class 2 'matchmakers'
- Link drug to a biological effector



VHL MZ1 $BRD4^{BD2}$


(a) Occupancy-driven pharmacology
Protein function is modulated *via* inhibition
(b) Event-driven pharmacology
Protein function is modulated *via* PROTAC induced degradation
NH2
26S proteasomal degradation
Catalytic process
POI
Small molecule inhibitor
E3 ligase
E2 enzyme
Ubiquitin
Peptide fragment
(c) PROteolysis TArgeting Chimera (PROTAC)
Linker
POI ligand
E3 ligase ligand
FKBP
FKBP-targeting PROTAC
N-ALAPYIP-(D-Arg)8-CONH2
HIF-1α peptide (VHL)
SARM
First all small molecule-based PROTAC
Nutlin (MDM2)
RIPK2
RIPK2-targeting PROTAC
VHL (VHL ligand)
BRD
BRD-targeting PROTAC
IMiD (CRBN)
Drug Discovery Today: Technologies

Offline Activities

- **Reading about RNA therapies:**
  - **Levin, Arthur A. 2019. “Treating Disease at the RNA Level with Oligonucleotides.” New England Journal of Medicine 380 (1): 57–70. https://doi.org/10.1056/NEJMra1705346.**
  - **Oligonucleotides and their discontents: https://blogs.sciencemag.org/pipeline/archives/2021/03/24/oligonucleotides-and-their-discontents**

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